

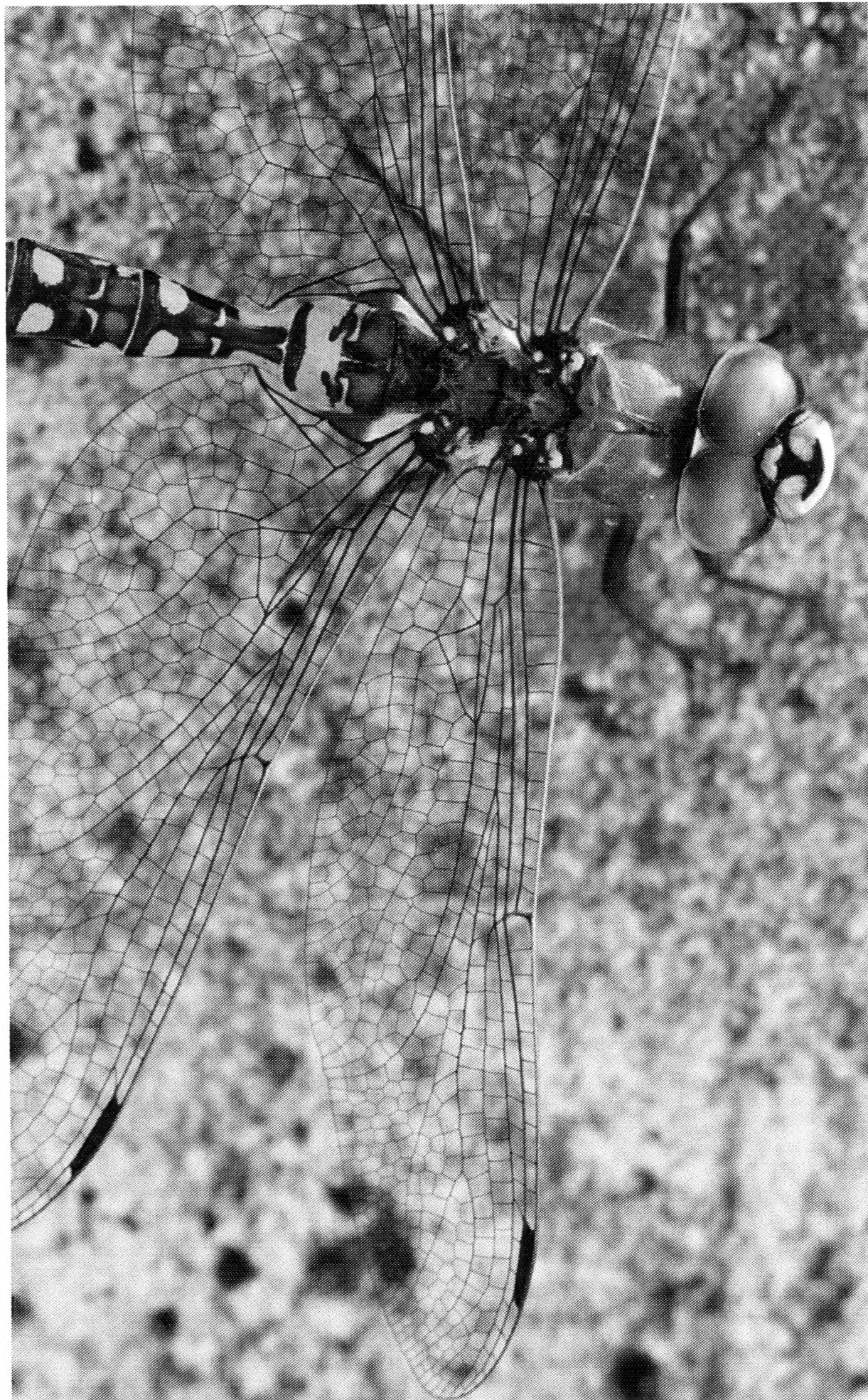
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COVER: *Aeshna spp*

Photographed on the UBC-O campus on a cold morning against a concrete retaining wall in the centre of the campus, by the fountain.

Photograph details:

Photograph by Robert Lalonde, University of British Columbia – Okanagan. The background is a concrete retaining wall in the centre of the campus, by the fountain. Canon Digital Rebel T2i; 100mm macro lens; no flash; f8 in aperture mode; shutter speed unknown.

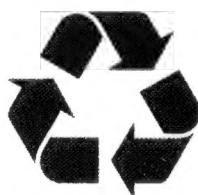
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Development and oviposition preference of *Xenotemna pallorana* Robinson (Lepidoptera: Tortricidae) on alfalfa and fruit tree foliage

C. A. NOBBS¹, R. S. PFANNENSTIEL², and J. F. BRUNNER³

ABSTRACT

Xenotemna pallorana Robinson (Lepidoptera: Tortricidae) is an alfalfa (*Medicago sativa* L., Fabaceae) feeding leafroller that has been considered for incorporation into apple (*Malus domestica* Borkh., Rosaceae) orchard ecosystems in Washington State, U.S.A., as an alternative host for the leafroller parasitoid *Colpoclypeus florus* Walker (Hymenoptera: Eulophidae). *Xenotemna pallorana* has been observed to feed on apple foliage when populations deplete the foliage of alfalfa in a groundcover, but there have been no studies to determine if foliage of fruit trees is suitable for full larval development or would be attractive as oviposition sites. Leafroller larvae were fed apple, cherry (*Prunus avium* L., Rosaceae), pear (*Pyrus communis* L., Rosaceae), and alfalfa foliage, all of which proved suitable for development; although development time and pupal weights varied among foliage types. Adult female *X. pallorana* exposed to apple foliage under no-choice conditions oviposited on the upper side of apple leaves. In a choice test between apple foliage and ground cover including alfalfa, *X. pallorana* females preferentially selected alfalfa and other components of the ground cover (98.04%) over the apple foliage (1.94%) for oviposition. Despite the ability of *X. pallorana* to develop on fruit tree foliage, its distinct preference for ovipositing on alfalfa suggests that it is unlikely to damage fruit. Therefore, *X. pallorana* presents a low-risk opportunity to study enhancement of biological control of leafrollers in orchards through ground cover management and host augmentation.

Key Words: *Colpoclypeus florus*, *Xenotemna pallorana*, ground cover, alternative host

INTRODUCTION

Once considered secondary or minor pests, the leafrollers *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) and *Pandemis pyrusana* Kearfott (Lepidoptera: Tortricidae) are now two of the most important pests in Washington pome fruit orchards (Brunner and Beers 1990; Brunner 1994; Brunner 1996b; Brunner 1999). This is especially true in orchards that use pheromone-based mating disruption for the control of codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) (Gut and Brunner 1998). The seasonal life histories of *C. rosaceana* and *P. pyrusana* are similar and fairly synchronous, with both species having two generations per year in Washington State and overwintering as small larvae (Beers *et al.* 1993). Control of leafrollers in orchards has historically relied on broad-spectrum insecticides (Beers *et al.* 1993; Brunner 1999); however, the development of insecticide resistance has made control difficult (Sial and Brunner 2010a). Additionally, even the integration of insecticides with newer chemistries might lead to resistance onset within 5 to 10 generations (Sial and Brunner 2010b) if not carefully managed. The loss of conventional control products and continued evolution of resistance to pesticides greatly increase the need for non-chemical alternatives to suppress leafroller populations in orchards.

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Biological control may play a significant role in an integrated approach for leafroller control. One natural enemy that has shown promise against leafrollers in Washington State is the gregarious ectoparasitoid *Colpoclypeus florus* Walker (Hymenoptera: Eulophidae). This species was first discovered in Washington State in 1992, where it parasitized about 80 percent of the summer generation *P. pyrusana* in an unsprayed apple orchard (Brunner 1996a). In Europe, *C. florus* parasitizes more than 30 species of tortricid larvae (Dijkstra 1986) and has been reported to parasitize large numbers of leafrollers in Europe that feed on both apple and strawberries (Gruys and Vaal 1984). However, it is absent or rare in spring, although it can be common in summer and early fall, causing significant mortality of leafroller larvae (Gruys 1982). The absence of *C. florus* in spring limits its impact as a biological control agent in Europe. The disparity in the seasonality of parasitism is presumably caused by a lack of synchrony between the phenology of *C. florus* and leafroller species present in European orchards (Gruys 1982). *Colpoclypeus florus* attacks relatively large leafroller larvae (Gruys and Vaal 1984; Dijkstra 1986); however, most tortricids found in orchard systems overwinter as early larval instars that are not suitable hosts for *C. florus* in Europe (Gruys and Vaal 1984). Female *C. florus* searching for suitable hosts in the fall within orchards cannot find them and evidently leave the orchard environment in search of suitable overwintering hosts (van Veen and Wijk 1987). In Europe, no leafrollers have been found that provide overwintering opportunities in orchards for *C. florus* (Evenhuis and Vlug 1983). However, if suitable hosts are placed within orchards, parasitism is high (Pfannenstiel *et al.* 2012).

Parasitism of the summer leafroller generation in Washington by *C. florus* can be high (>60%) (Brunner 1996a), but spring parasitism is generally low, and parasitism of the summer generation is often insufficient to prevent fruit damage (Unruh *et al.* 2001). As in Europe, the leafroller pests in Washington orchards, *C. rosaceana* and *P. pyrusana*, overwinter as instars that are typically too small to be suitable hosts for *C. florus* (Pfannenstiel and Unruh 2003; Pfannenstiel *et al.* 2010). One possible tactic to augment biological control of leafrollers would be to provide alternate overwintering hosts for *C. florus*.

A leafroller being considered for this is *Xenotemna pallorana* Robinson (Lepidoptera: Tortricidae). This leafroller is an alfalfa-feeding species that is in the same tribe (Archipini) as both *C. rosaceana* and *P. pyrusana*, and may overwinter in an appropriate stage for *C. florus* to attack in the fall. *Xenotemna pallorana* is common in alfalfa crops in some parts of the Columbia Basin (CAN and RSP personal observation). Alfalfa occurs in orchard groundcovers in Central Washington, but currently *X. pallorana* is not commonly observed in orchards (RSP personal observation). Establishment of *X. pallorana* in orchard groundcovers in the fall would allow *C. florus* to locate hosts for overwintering within the orchard and, thus, be more abundant in orchards the following spring. *Xenotemna pallorana* is bivoltine and highly polyphagous (Chapman and Lienk 1971), having been reported feeding on rose and birdsfoot trefoil in New York (Schott 1925; Neunzig and Gyrisco 1955), young white pine stands in Michigan (McDaniel 1936), strawberries in Ohio (Neiswander 1944), and seed alfalfa in Utah (Snow and McClellan 1951).

Preliminary evaluations demonstrated that *X. pallorana* is a suitable host for *C. florus* (CAN and RSP, unpublished data). When host foliage is depleted, *X. pallorana* will feed on apple leaves on young trees where foliage is near the ground (Chapman and Lienk 1971). The occurrence of *X. pallorana* in Washington apple orchards is associated with mature larvae using foliage for pupation sites (JFB personal observation).

Before *X. pallorana* can be recommended as a potential alternative host for *C. florus* in orchard ground covers, it is necessary to demonstrate that it would not consistently feed on and damage fruit trees. Our goal in this study is to determine whether *X. pallorana* feed and develop normally on foliage of three tree fruit species, whether adults oviposit on apple foliage under no-choice conditions, and finally whether *X. pallorana*

prefers to oviposit on apple tree foliage when an alfalfa-dominated ground cover is available.

MATERIALS AND METHODS

A laboratory colony of *X. pallorana* was initiated from individuals collected from alfalfa in the Columbia Basin near Quincy, WA, in June 1996. *Xenotemna pallorana* larvae were reared in groups (8) within 118-ml plastic portion cups (Solo®), on a modified pinto bean-based diet (Shorey and Hale 1965) at 24 ± 2 °C with a 16:8 (L:D) photoperiod. Pupae were collected and placed into a cylindrical wire mesh oviposition cage 20 cm tall and 10 cm in diameter. Waxed paper was placed into oviposition cages, and *X. pallorana* was allowed to eclose, mate and oviposit on the waxed paper. Exposure to natural light greatly increased oviposition, so oviposition cages were positioned near windows in the rearing rooms. Egg masses were cut from the waxed paper, washed in a 5% sodium hypochlorite solution, and then rinsed with distilled water. Eggs were carefully peeled from the waxed paper and placed into petri dishes (Falcon 5009, 50 x 9 mm), with a 1-cm cube of artificial diet. Neonates were transferred in groups of eight to 104-ml portion cups, with the bottoms filled approximately 1 cm deep with artificial diet for larval development.

Development on fruit tree hosts. Forty newly hatched *X. pallorana* larvae were placed individually into Petri dishes (Falcon 5009, 50 x 9 mm) containing an approximate 4-cm² portion of a mature leaf from a growing shoot (4 to 10 leaves from shoot apex) of apple (*Malus domestica* Borkhausen var. Red Spur Delicious), pear (*Pyrus communis* L. var. Bartlett), or cherry (*Prunus avium* L. var. Bing). For alfalfa, the apical 4 to 5 leaves of a non-blooming shoot were collected and placed in the same arena type as fruit tree foliage. Our goal here was simple; to see if the larvae would complete development to adult on the different foliage types. The use of cut foliage, which can be lower in quality, provides a conservative estimate of suitability and allowed the study to be conducted under controlled conditions. Conducting this study on growing trees in the orchards would have exposed larvae to significant environmental variation and mortality that might have masked the effects of foliage type and prevented estimation of plant-based survival rates.

Fruit tree foliage was obtained from multiple trees for each type within unsprayed orchards at the Washington State University Tree Fruit Research and Extension Center (WSU-TFREC) in Wenatchee, WA. Alfalfa foliage was obtained from plants transplanted from the field to 3.8-l pots in the greenhouse containing 3-l of Sunshine potting mix #1 (Sungro Horticulture, Agawam, MA, USA) with 30 ml of long-release fertilizer granules spread over the surface (Osmocote, Everris NA). *Xenotemna pallorana* larvae were placed in a controlled environment at 24 ± 2 °C, 70 ± 10 % RH, and 16:8 (L:D) photoperiod. Larvae were examined daily, and mortality was recorded. Food was changed as needed, when it had been consumed, or if a decline in apparent quality was observed, but no less than twice per week. Pupating larvae were set aside and weighed within 48 h of pupal formation but after they had completed melanization. Chi-square analysis was used to determine if food source affected survivorship of *X. pallorana*. The sex of newly emerged adults was recorded. Larval and pupal development time and pupal weights for each sex reared on each foliage type were analyzed using one-way analysis of variance (ANOVA; Super ANOVA general linear model program; Abacus Concepts, Berkeley, CA). Mean separations were done with the Fisher protected least significant difference test (LSD $\alpha = 0.05$).

Oviposition Experiments. Experiments were conducted to determine whether *X. pallorana* would oviposit on apple foliage in choice and no-choice situations. For the no-choice studies, 12 sleeve cages made of cloth and wire screen (window screen tubes, approximately 50 cm long and 25 cm in diameter) were placed over unsprayed apple (c.v. Red Spur) foliage in the field. Newly emerged male and female *X. pallorana* adults

(<12h old, two of each) obtained from the laboratory colony were released into each cage. After a period of seven days, each cage was removed, all egg masses were collected, and their locations were recorded.

A second experiment was conducted to determine if *X. pallorana* females prefer to oviposit on foliage of a fruit tree host, apple, or their typical field host, alfalfa, as part of a groundcover. Four nylon organdy mesh cages (1.22 m x 1.22 m x 1.22 m) were suspended from a frame of plastic irrigation pipe (PVC, 2 cm) and placed over patches of alfalfa plants in a fescue (*Festuca* spp; Poaceae) dominated groundcover. A single potted apple tree (c.v. "Oregon Spur", ~ 1 m tall, with at least 30 fully developed leaves) was placed in each cage. Alfalfa typically comprised <50% of the ground cover foliage. Although the leaf area was not directly measured for each foliage type, alfalfa foliage made up a smaller proportion of the potential oviposition substrates than the apple and fescue foliage (CAN and RSP personal observation). Ten newly emerged colony-reared *X. pallorana*, (1:1 sex ratio) were released into each cage and allowed to mate and oviposit for seven days. Seven days after the moths were released, cages were removed, and egg masses were collected from foliage within each cage ($n=12$). The proportion of egg masses deposited on apple foliage vs. alfalfa and other groundcover plants was compared to the null hypothesis of no preference (50:50) using Chi-square analysis.

RESULTS AND DISCUSSION

A similar number of *X. pallorana* survived to adulthood when reared on the various foliage types ($\chi^2 = 3.42$, $df = 3$, $P = 0.3313$). Survival of *X. pallorana* on the factitious hosts was 65% on cherry, 72.5% on pear, and 75% on apple foliage. Survival on alfalfa was the lowest overall, at 56%; most likely, this is an artifact related to the quality of the alfalfa foliage declining more quickly than the apple foliage in the bioassay arena. The host material that larvae were reared on significantly affected larval developmental time for both males and females ($F = 16.155$; $df = 3$; $P < 0.0001$ and $F = 7.647$; $df = 3$; $P = 0.0003$, respectively), whereas pupation time was not different (Table 1). There was also a significant effect of host material on pupal weight for both males and females ($F = 57.477$; $df = 3$; $P < 0.0001$ and $F = 43.864$; $df = 3$; $P < 0.0001$, respectively; Table 2). *Xenotemna pallorana* was able to complete its life cycle on the foliage of apple, cherry, and pear, in addition to alfalfa. Larval development was fastest on alfalfa. Pupal weights were consistently higher for larvae reared on apple and cherry foliage than on alfalfa and pear. Adults from the heaviest pupae (reared on apple and cherry foliage) had unusually large abdomens in proportion to their wing size and had difficulty flying (CAN and RSP, personal observation). It would have been difficult for these adults to disperse and either mate or deposit egg masses. It may be that the larvae developing on apple and cherry develop to such a large size through a supernumerary instar, which would account for both the longer development time and the larger pupal size. Adults that developed on alfalfa and pear foliage had physical proportions similar to those of individuals collected from the field or reared on artificial diet.

In no-choice trials, *X. pallorana* readily oviposited on apple foliage. Egg masses were found on apple foliage in 8 of 12 sleeve cages, with an average of 2.08 egg masses per cage. These egg masses successfully hatched, and the larvae were observed to begin feeding on the leaves; however, further development was not monitored. Egg masses were found only on the upper surface of apple leaves. Therefore, without a choice, *X. pallorana* will accept apple foliage for oviposition. Because *X. pallorana* oviposits on wax paper in the laboratory and has been observed to lay egg masses on other smooth material such as glass or plastic, oviposition on apple foliage when confined was not surprising.

Table 1
Development time of *X. pallorana* reared on different hosts.

Host	Sex	n	Larval developmental time (mean days ± SEM)	Pupation time (mean days ± SEM)
Cherry	Male	13	35.6 ± 1.1a	11.9 ± 0.2a
Pear		21	32.0 ± 0.9b	11.8 ± 0.2a
Apple		11	34.4 ± 1.2ab	11.9 ± 0.3a
Alfalfa		14	25.8 ± 1.1c	11.9 ± 0.2a
Cherry	Female	13	40.8 ± 1.1a	11.1 ± 0.2a
Pear		8	36.5 ± 1.4b	10.9 ± 0.3a
Apple		19	35.8 ± 0.9b	10.8 ± 0.2a
Alfalfa		9	33.1 ± 1.3b	10.2 ± 0.3a

Means within a column for the same sex followed by the same letter are not significantly different ($P > 0.05$); Fisher’s protected LSD test.

Table 2
Pupal weight of *X. pallorana* reared on different hosts.

Host	Sex	n	Pupal weight (g) (mean ± SEM)
Cherry	Male	13	0.055 ± 0.002b
Pear		22	0.038 ± 0.002d
Apple		11	0.073 ± 0.002a
Alfalfa		14	0.047 ± 0.002c
Cherry	Female	13	0.058 ± 0.004b
Pear		7	0.044 ± 0.005c
Apple		19	0.094 ± 0.003a
Alfalfa		9	0.042 ± 0.005c

Means within a column for the same sex followed by the same letter are not significantly different ($P > 0.05$); Fisher’s protected LSD test.

In choice trials, *X. pallorana* were presented with apple trees and alfalfa in a fescue-dominated ground cover. A total of 51 egg masses were collected (Table 3), with an average of 4.25 egg masses per cage ($n = 12$). The number of egg masses found on alfalfa and other ground cover plants was significantly higher than on apple, with slightly over 98% of the egg masses (50) found on alfalfa and other ground cover plants (74.5% on alfalfa and 23.5% on other ground cover plants; $\chi^2 = 31.5$, $df = 1$, $P << 0.0001$) when compared to the null hypothesis of no preference. Only one egg mass was found on apple foliage—less than 2% of the total collected.

Table 3

Oviposition choice of *X. pallorana* females between apple foliage and an alfalfa-dominated cover crop.

Foliage type	Total # egg masses collected	Percent of total	Average # per cage
Apple	1	2.0	0.08
Other	12	23.5	1.00
Alfalfa	38	74.5	3.17

Therefore, when provided a choice, *X. pallorana* females predominantly deposited their eggs on alfalfa and other groundcover plants over apple foliage. Other than alfalfa, ground cover plants on which egg masses were found included blades of fescue (Poaceae); dandelion (*Taraxacum officinale* Weber, Asteraceae); and field bindweed (*Convolvulus arvensis* L, Convolvulaceae). The single egg mass found on apple, 2% of the total egg masses, may have been the result of the location of foliage in close proximity to the ground cover. *Xenotemna pallorana* will lay eggs on apple foliage under no-choice situations. The species can be locally abundant in alfalfa fields in the Columbia Basin, but are not observed frequently in orchards (CAN and RSP, personal observation). Oviposition preference for alfalfa most likely explains why *X. pallorana*, although occurring in fruit growing areas of Washington and commonly observed in alfalfa fields, is not commonly observed feeding on apple or other fruit trees. Additionally, the density of alfalfa in eastern Washington apple orchards varies considerably, and most orchards use pesticides for insect control, making observations of *X. pallorana* less likely.

In conclusion, *X. pallorana* can complete development on apple, cherry, and pear foliage, as well as it can on alfalfa, although developmental time is longer in most cases. Although females may oviposit almost exclusively on a limited range of hosts, they may develop as fast and as well on non-preferred hosts (Thompson 1988). There is no aversion of females to oviposition on apple foliage when not given a choice, but under natural conditions it is clear that they preferentially select ground cover habitats for oviposition sites. It may be that *X. pallorana* doesn't need to deposit eggs exclusively on hosts such as alfalfa as long as suitable hosts are nearby, which may explain the frequency of oviposition on non-alfalfa groundcover plants. Larvae may search for and disperse to alfalfa in groundcover to some extent following hatch. Although this research shows that at least three orchard crops are suitable hosts for *X. pallorana*, we believe the strong oviposition preference for alfalfa would preclude any pest potential by this species. The *X. pallorana*–alfalfa combination may offer a low-risk model for the orchard environment to test the hypothesis that cover-crop management could be used to enhance leafroller biological control.

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Efficacy of SPLAT® Verb for protecting individual *Pinus contorta*, *Pinus ponderosa*, and *Pinus lambertiana* from mortality attributed to *Dendroctonus ponderosae*

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ABSTRACT

Verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) is an antiaggregant of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae), the most notable forest insect pest in western North America. Several formulations are registered for tree protection, but efficacy is often inconsistent. We evaluated the efficacy of a newly registered formulation of (–)-verbenone (SPLAT® Verb, ISCA Technologies Inc., Riverside, CA, USA) for protecting individual lodgepole pines, *Pinus contorta* Dougl. ex Loud, ponderosa pines, *P. ponderosa* Dougl. ex Laws., and sugar pines, *P. lambertiana* Dougl., from mortality attributed to *D. ponderosae*. Rather than a single release device, SPLAT® Verb is a flowable emulsion that allows the user to adjust the size of each release point (dollop) according to desired rates and distributions. SPLAT® Verb applied at 7.0 g of (–)-verbenone/tree as four equally sized dollops to the tree bole was effective for protecting *P. contorta*, but not *P. ponderosa*. In *P. lambertiana*, 4.0, 7.0, and 10.0 g of (–)-verbenone/tree were effective. We discuss the implications of these and other results to the management of *D. ponderosae*.

Key Words: mountain pine beetle, Scolytinae, semiochemicals, tree protection, verbenone

INTRODUCTION

Mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae), is a major disturbance agent in conifer forests of western North America, where it colonizes at least 15 native pines, most notably lodgepole, *Pinus contorta* Dougl. ex Loud., ponderosa, *P. ponderosa* Dougl. ex Laws., sugar, *P. lambertiana* Dougl., limber, *P. flexilis* E. James, western white, *P. monticola* Dougl. ex D. Don, and whitebark, *P. albicaulis* Engelm., pines (Negrón and Fettig 2014). The geographic distribution of *D. ponderosae* ranges from British Columbia, Canada, east to South Dakota, United States, and south to Baja California, Mexico. Populations have recently been reported in Nebraska, United States (Costello and Schaupp 2011), and the insect is expanding its range northward in British Columbia and eastward in Alberta, Canada (de la Giroday *et al.* 2012). In the last decade, outbreaks of *D. ponderosae* have impacted > 27 million hectares of forest (USDA Forest Service 2012; British Columbia

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Ministry of Forests, Lands and Natural Resource Operations 2013), and will continue to be important forest disturbances—particularly given the magnitude of warming projected for western North America, and its direct and indirect effects on *D. ponderosae* (Carroll *et al.* 2003; Bentz *et al.* 2010). In conjunction with projected warming trends, susceptible forest landscapes still exist throughout western North America. Although *D. ponderosae* is native to North America and an important part of the ecology of North American forests, tree mortality resulting from outbreaks may have undesirable social–ecological impacts, for example, negatively affecting aesthetics, recreation, fire risk and severity, human safety, timber production, wildlife habitat, and real estate values, among many other resources.

Progar *et al.* (2014) provided a thorough review of the chemical ecology of *D. ponderosae* relevant to host finding, selection, colonization, and mating behaviors. In short, females initiate colonization of the lower tree bole in a behavioral sequence mediated by aggregation pheromones (Vité and Gara 1962; Pitman *et al.* 1968, 1969; Ryker and Libbey 1982) and host kairomones (Renwick and Vité 1970; Borden *et al.* 1987; Miller and Lindgren 2000). Females are subsequently joined by males, and mass attack ensues (Pitman *et al.* 1968), enabling *D. ponderosae* to overwhelm host tree defenses consisting of anatomical and chemical components that are both constitutive and inducible (Franceschi *et al.* 2005). During latter stages of colonization, increasing amounts of verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) are produced (Pitman *et al.* 1969; Rudinsky *et al.* 1974), which inhibit additional *D. ponderosae* from infesting the target tree. The first evidence of this effect was documented by Ryker and Yandell (1983) in laboratory and field assays. In nature, verbenone is produced in small amounts by autoxidation of the monoterpene α -pinene (Hunt *et al.* 1989), but the principal route of production is through metabolic conversion by bark beetles of inhaled and ingested α -pinene to the terpene alcohols *cis*- and *trans*-verbenol, which are then metabolized to verbenone by yeasts in the alimentary system and within beetle galleries (Hunt and Borden 1990). It is assumed that verbenone reduces intraspecific competition and, perhaps, interspecific competition, by altering adult behavior to minimize overcrowding of developing brood within the host (Byers and Wood 1980). Lindgren *et al.* (1996) proposed that verbenone is an indicator of host tissue quality and that its quantity is a function of microbial degradation.

Fettig *et al.* (2014) discussed approaches for reducing the negative impacts of *D. ponderosae* on forests. Direct control involves short-term tactics designed to address current infestations by manipulating beetle populations, and commonly includes the use of insecticides, semiochemicals (i.e., chemicals produced by one organism that elicit a behavioral response in another organism), sanitation harvests, or a combination of these treatments. The use of semiochemicals has largely focused on verbenone for protecting individual, high-value trees or small groups of trees (e.g., in campgrounds). Results have been favorable, but inconsistent (for a detailed explanation of associated factors, see Progar *et al.* 2014). While several formulations of verbenone are registered for use in Canada and the United States, pouches (several registrants) stapled at maximum reach (~2 m in height) to individual trees or applied in a grid pattern for stand protection are most commonly used (Gillette and Munson 2009).

Fettig *et al.* (2015) recently developed a novel formulation of (–)-verbenone (SPLAT® Verb, ISCA Technologies Inc., Riverside, CA, USA) for protecting individual *P. contorta* and stands of *P. contorta* from mortality attributed to *D. ponderosae*. Rather than a single release device such as the pouch, SPLAT® Verb is a flowable emulsion that allows the user to adjust the size of each release point (dollop) according to desired distributions in the field. SPLAT® Verb is a “matrix-type” diffusion controlled-release device specifically designed to release (–)-verbenone over a sustained period (~8–24 wks, depending on dollop size) at rates suitable to provide significant reductions in levels of *P. contorta* mortality at relatively low doses (Mafra-Neto *et al.* 2013). Dollops biodegrade within ~1 yr of application and, as such, do not need to be retrieved from the

field as do most other release devices used to dispense verbenone. SPLAT® Verb was registered by the United States Environmental Protection Agency (USEPA) for use on pines, *Pinus* spp., in August 2013, and was first used commercially in the United States in 2014. The objective of our research was to determine the efficacy of SPLAT® Verb for protecting individual *P. contorta*, *P. ponderosa*, and *P. lambertiana* from mortality attributed to *D. ponderosae*.

MATERIALS AND METHODS

Studies were conducted in three locations (see below) selected based on aerial and ground surveys that indicated *D. ponderosae* was causing noticeable levels of tree mortality in each area. Experimental trees were treated according to the criteria described below for each study. Regardless of treatment, one commercially available two-component tree bait [*trans*-verbenol (~1.2 mg/d) and *exo*-brevicomin (~0.3 mg/d); Contech Inc., Delta, BC, Canada] was stapled to the bole of each experimental tree immediately after treatment at ~2 m in height on the northern aspect, and left in place until beetle flight had ceased (dates reported in Tables 1–3). The manufacturer estimates the life expectancy of these baits is 100–150 days, depending on weather conditions, covering most of the flight activity period of *D. ponderosae* at each location.

Initially, success of *D. ponderosae* attacks was based on visual assessments of pitch tubes and boring dust (condition, distribution, and density) during August–September of the year treatments were implemented. At that time, experimental trees were recorded as not attacked, unsuccessfully attacked, strip attacked, or mass attacked (Gibson *et al.* 2009). This allows for a surrogate estimate of treatment efficacy should the experimental infrastructure be compromised or lost (e.g., due to wildfire, which is common in the western USA). However, tree mortality was ultimately based on presence (dead) or absence (live) of crown fade ~1 yr after treatments were implemented, except for Experiment 3, when evaluations were conducted in late-September of the same year. The only criterion used in determining the effectiveness of each treatment was whether individual trees died due to colonization by bark beetles. Treatments were considered to have experienced sufficient beetle “pressure” (i.e., a relative measure of population density based on levels of tree mortality) to permit determination of efficacy if $\geq 60\%$ of the untreated control trees were killed by bark beetles. SPLAT® Verb treatments were considered efficacious if < 7 trees were killed by bark beetles (Hall *et al.* 1982; Shea *et al.* 1984). These criteria were established based on a sample size of 22–35 trees and test of the null hypothesis, $H_0: S$ (survival $\geq 90\%$). These parameters provide a conservative binomial test ($\alpha = 0.05$) to reject H_0 when > 6 trees die. The power of this test, that is the probability of having made the correct decision in rejecting H_0 , is 0.84 (Hall *et al.* 1982; Shea *et al.* 1984). This experimental design provides a very conservative test of efficacy, and was originally developed for evaluating the efficacy of bole-applied insecticides to protect individual trees from bark beetle attack. When properly applied, insecticides typically provide higher levels of tree protection than verbenone (Fettig *et al.* 2013; Progar *et al.* 2014), and as such, our experimental design represents a rigorous examination of the efficacy of SPLAT® Verb.

Experiment 1 – *Pinus contorta*: This study was conducted on the Wisdom Ranger District, Beaverhead–Deerlodge National Forest, Montana, United States (45° 24' 28.98" N, 113° 39' 28.92" W; 2150 m elevation) during 2013/2014. Surrounding stands had a mean live tree (≥ 12.7 cm dbh; diameter at 1.37 m in height) density of 18.0 m²/ha of basal area (cross-sectional area of trees at 1.37 m in height), of which 87.2% was *P. contorta* with a mean quadratic mean diameter (QMD, the diameter corresponding to mean basal area) of 20.7 cm. The remainder was represented by Engelmann spruce, *Picea engelmannii* Parry ex Engelm., and subalpine fir, *Abies lasiocarpa* (Hooker) Nuttall. About 28.8% of *P. contorta* and 43.1% of *P. contorta* basal area had been killed by *D. ponderosae* during the two years preceding the study (cause of death and time

since death determined by gallery patterns in the phloem, and by color and needle retention of the crown, respectively; Klutsch *et al.* 2009).

Thirty trees (min. dbh = 18.5 cm) were confirmed uninfested and randomly assigned to each of two treatments ($N = 60$): (1) SPLAT® Verb [10.0% (–)-verbenone by weight; EPA Reg. No. 80286–20] applied at 7.0 g of (–)-verbenone/tree (70 g of SPLAT® Verb/tree) as four 17.5-g dollops (~5.5 cm diam. X 1.2 cm ht.) to the tree bole at cardinal directions at ~2.5 m in height using a caulking gun (Model X-Lite, Newborn Brothers Co., Inc., Jessup, MD, USA); and (2) an untreated control. Treatments were applied on 29–30 June 2013. Adjacent experimental trees were separated by ≥ 100 m. There was no significant difference in tree dbh between treatments ($F_{1, 58} = 0.3$, $P = 0.60$; Table 1), a factor that influences tree susceptibility to *D. ponderosae* (Shepherd 1966). The integrity of dollops was visually inspected 18–19 September 2013 for evidence of contact or consumption by animals.

Table 1

Efficacy of SPLAT® Verb (ISCA Technologies Inc., Riverside, CA, USA) for protecting individual *Pinus contorta* from mortality attributed to *Dendroctonus ponderosae*, Wisdom Ranger District, Beaverhead–Deerlodge National Forest, Montana (45° 24' 28.98" N, 113° 39' 28.92" W; 2150 m elevation), 2013/2014.

Treatment	Dose ^a	Mean dbh ± SEM	Mortality/n
Untreated control	0	26.2 ± 0.7	26/30
SPLAT® Verb	7	26.8 ± 0.9	2/30

^a Values are grams of (–)-verbenone applied as four 17.5-g dollops (~5.5 cm diam. X 1.2 cm ht.) to the tree bole at cardinal directions at ~2.5 m in height using a caulking gun. One tree bait (Contech Inc., Delta, BC, Canada) was attached to the bole of each tree at ~2 m in height on the northern aspect 29–30 June to 18–19 September 2013.

Experiment 2 – *Pinus ponderosa*: This study was conducted on the Darby Ranger District, Bitterroot National Forest, Montana, United States (46° 04' 22.0" N, 114° 14' 17.7" W; 1344 m elevation) during 2013/2014. Surrounding stands had a mean live tree (≥ 12.7 cm dbh) density of 27.2 m²/ha of basal area, of which 92.7% was *P. ponderosa* with a mean QMD of 38.9 cm. The remainder was represented by Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franc. About 20.8% of *P. ponderosa* and 12.4% of *P. ponderosa* basal area had been killed by bark beetles, primarily *D. ponderosae*, during the two years preceding the study.

Thirty trees (min. dbh = 21 cm) were confirmed uninfested and randomly assigned to each of the two treatments described in Experiment 1 ($N = 60$). Treatments were applied on 30 June–1 July 2013. Adjacent experimental trees were separated by ≥ 100 m. There was no significant difference in tree dbh between treatments ($F_{1, 58} = 0.7$, $P = 0.40$; Table 2).

Experiment 3 – *Pinus lambertiana*: This study was conducted on the Groveland Ranger District, Stanislaus National Forest, California, United States (37° 49' 48.63" N, 119° 51' 19.44" W; 1417 m elevation) during 2014/2015 in areas impacted by the Rim Fire. The Rim Fire is the largest wildfire on record in the Sierra Nevada, California, and burned 104,131 ha in August 2013 (Kirn and Dickman 2013). The study area was impacted by mixed-severity fire, with ~26.1% of trees (all species) killed by fire, mostly in the smaller-diameter classes (< 31.8 cm dbh). Surrounding stands had a mean live tree (≥ 16.5 cm dbh) density of 54.0 m²/ha of basal area, of which 30.7% was *P. lambertiana* with a mean QMD of 75.4 cm. The remainder was represented by white fir, *A. concolor*

(Gordon) Lindley ex Hildebrand, *P. ponderosa*, incense cedar, *Calocedrus decurrens* (Torr.) Florin, and, to a much lesser extent, black oak, *Quercus kelloggii* Newb. About 5.1% of *P. lambertiana* died during the two years preceding the study, most of which was attributed to fire, although several trees were colonized and killed by *D. ponderosae*. Despite the low level of beetle “pressure” observed, substantial increases in levels of tree mortality attributed to *D. ponderosae* were expected in 2014 due to the confounding effects of fire on tree susceptibility to colonization by *D. ponderosae* (Jenkins *et al.* 2014).

Table 2
Efficacy of SPLAT® Verb (ISCA Technologies Inc., Riverside, CA, USA) for protecting individual *Pinus ponderosa* from mortality attributed to *Dendroctonus ponderosae*, Darby Ranger District, Bitterroot National Forest, Montana (46° 04' 22.0" N, 114° 14' 17.7" W; 1344 m elevation), 2013/2014. Some trees were also colonized by *Dendroctonus brevicomis*, *Ips pini*, and *I. emarginatus*.

Treatment	Dose ^a	Mean dbh ± SEM	Mortality/n
Untreated control	0	32.1 ± 1.0	28/29 ^b
SPLAT® Verb	7	30.8 ± 1.2	9/30

^a Values are grams of (–)-verbenone applied as four 17.5-g dollops (~5.5 cm diam. X 1.2 cm ht.) to the tree bole at cardinal directions at ~2.5 m in height using a caulking gun. One tree bait (Contech Inc., Delta, BC, Canada) was attached to the bole of each tree at ~2 m in height on the northern aspect 30 June–1 July to 17–18 September 2013.
^b One tree could not be located, and presumably was removed by woodcutters.

Twenty-five trees (min. dbh = 29 cm) were confirmed uninfested and randomly assigned to each of four treatments (*N* = 100): (1) SPLAT® Verb applied at 4.0 g of (–)-verbenone/tree (40 g of SPLAT® Verb/tree) as four 10.0-g dollops (~4.5 cm diam. X 1.5 cm ht.) to the tree bole at cardinal directions at ~2.5 m in height, (2) SPLAT® Verb applied as in Experiments 1 and 2, (3) SPLAT® Verb applied at 10.0 g of (–)-verbenone/tree (100 g of SPLAT® Verb/tree) as four 25.0-g dollops (~5.7 cm diam. X 2.8 cm ht.) to the tree bole at cardinal directions at ~2.5 m in height, and (4) an untreated control (Table 3). Treatments were applied 23–24 May 2014. Adjacent experimental trees were separated by ≥ 50 m. There was no significant difference in tree dbh (*F*_{3, 96} = 0.5, *P* = 0.71) or percent crown volume scorched (*F*_{3, 96} = 0.5, *P* = 0.71) among treatments (Table 3). The latter is a significant predictor of the probability of *P. lambertiana* mortality following fire (Hood *et al.* 2010). Although not well studied in *P. lambertiana*, pines injured by fire are more susceptible to colonization by *D. ponderosae* (Jenkins *et al.* 2014).

RESULTS

Dendroctonus ponderosae “pressure” was sufficient to adequately challenge treatments as 87%, 93%, and 72% of untreated, baited *P. contorta*, *P. ponderosa*, and *P. lambertiana*, respectively, died from colonization by bark beetles. SPLAT® Verb was effective for protecting *P. contorta* and *P. lambertiana* (tables 1 and 3) from mortality attributed to *D. ponderosae*, but not *P. ponderosa* (Table 2). All doses evaluated for protection of *P. lambertiana* were efficacious (Table 3).

Table 3

Efficacy of SPLAT® Verb (ISCA Technologies Inc., Riverside, CA) for protecting individual *Pinus lambertiana* from mortality attributed to *Dendroctonus ponderosae*, Groveland Ranger District, Stanislaus National Forest, California (37° 49' 48.63" N, 119° 51' 19.44" W; 1417 m elevation), 2014/2015.

Treatment	Dose ^a	Mean dbh ± SEM	Percent crown volume scorched ± SEM	Mortality/n
Untreated control	0	62.0 ± 4.1	37.8 ± 6.0	18/25
SPLAT® Verb	4	58.6 ± 3.9	26.6 ± 4.9	6/25
SPLAT® Verb	7	60.4 ± 4.7	27.0 ± 4.6	4/25
SPLAT® Verb	10	66.2 ± 6.2	21.8 ± 4.4	1/25

^a Values are grams of (–)-verbenone applied as four equally sized dollops to the tree bole at cardinal directions at ~2.5 m in height using a caulking gun. One tree bait (Contech Inc., Delta, BC, Canada) was attached to the bole of each tree at ~2 m in height on the northern aspect 23–24 May to 27 August 2014.

DISCUSSION

Experiment 1 confirms results of an earlier study conducted in Wyoming, United States, demonstrating the efficacy of four 17.5-g dollops of SPLAT® Verb [7.0 g of (–)-verbenone/tree] for protecting individual *P. contorta* from *D. ponderosae* (Fettig *et al.* 2015). In comparison, two pouches [13.5–15.0 g of (–)-verbenone/tree, depending on manufacturer] are recommended per tree (Kegley and Gibson, 2009; Kegley *et al.* 2010) and generally provide ≥ 80% protection of *P. contorta* and *P. albicaulis*. For larger trees (> 61 cm dbh), three to four pouches may be used. One registrant suggests using more than six pouches: two pouches at ~1.5 and ~2.5 m in height on the northern aspect of individual trees, with additional pouches placed at 4–5 m intervals on vertical substrates around the treated tree. Alternatively, Fettig *et al.* (2015) reported that SPLAT® Verb provided complete (100%) protection of individual *P. contorta* at much lower doses and recommended applying four 17.5-g dollops/tree (i.e., one dollop placed at maximum reach at each cardinal direction). They attributed the high level of tree protection observed in their study to multiple release points per tree (Gillette *et al.* 2006) and the larger zone of inhibition (i.e., demonstrated to be at least 8 m in radius in trapping assays) provided by SPLAT® Verb when compared to other formulations of verbenone that have been studied (Miller 2002; Fettig *et al.* 2009a, 2015). Fettig *et al.* (2015) reported significantly fewer *P. contorta* (percentage of trees) killed on 0.041-ha circular plots surrounding *P. contorta* treated with SPLAT® Verb compared to untreated trees, suggesting attraction was disrupted at levels sufficient to impart tree protection within 11 m of the point of release. Although we did not measure this variable in Experiment 1, we observed larger numbers of *P. contorta* killed by *D. ponderosae* within the vicinity (~10 m) of untreated, baited controls compared to SPLAT® Verb-treated trees.

As observed in Experiment 2, others have reported verbenone is ineffective for reducing levels of *P. ponderosa* mortality attributed to *D. ponderosae* (e.g., Bentz *et al.* 1989; Lister *et al.* 1990; Gibson *et al.* 1991; Gibson and Kegley 2004; Negrón *et al.* 2006). However, to our surprise, several of the trees in Experiment 2 were also colonized by western pine beetle, *D. brevicomis* LeConte, pine engraver, *Ips pini* Say, and emarginated ips, *I. emarginatus* (LeConte), including eight of nine SPLAT® Verb-treated trees that died. These bark beetle species are capable of causing tree mortality and are not inhibited by (–)-verbenone at levels sufficient to impart tree protection (e.g., Devlin and Borden 1994; Fettig *et al.* 2009a,b). As such, another evaluation of the efficacy of

SPLAT® Verb for protecting *P. ponderosa* from mortality attributed to *D. ponderosae* should be considered in areas where other bark beetles, specifically *D. brevicornis*, are absent. This also serves as a reminder of the importance of carefully confirming the cause of tree mortality when developing semiochemical-based technologies, many of which impart species- or genera-specific responses.

Experiment 3 represents the first evaluation of a semiochemical-based tool for protecting *P. lambertiana* from *D. ponderosae*. Doses as low as 40.0 g of SPLAT® Verb/tree were efficacious (Table 3), suggesting lower doses may yield efficacy for protection of *P. contorta* and should be evaluated. For decades, populations of *P. lambertiana* have been heavily impacted by *Cronartium ribicola* J.C. Fisch, the exotic pathogen that causes white pine blister rust (Maloney *et al.* 2011). Although white pine blister rust can be fatal to all species of white pine, a gene is present at low frequency in *P. lambertiana* that confers immunity from *C. ribicola* (Kinloch *et al.* 1970). This gene controls a hypersensitive response in needles that prevents further fungal growth (Kinloch and Littlefield 1976). Restoring populations of *P. lambertiana* involves, among other factors, identifying white pine blister rust-resistant trees in the field and, where feasible, protecting these individuals from colonization by *D. ponderosae* with insecticides, particularly when epidemics occur. This is followed by selective breeding of these individuals, and eventual outplanting of white pine blister rust-resistant seedlings. SPLAT® Verb represents a more portable and less toxic alternative to insecticides for protecting white pine blister rust-resistant *P. lambertiana*, other high-value *P. lambertiana* (e.g., those growing in residential, recreational, and administrative sites), or *P. lambertiana* that might otherwise be experiencing short-term stressors that increase susceptibility to colonization by *D. ponderosae*. Relatedly, in 2014 we were asked to treat several large-diameter, fire-injured *P. lambertiana* in areas impacted by the Rim Fire within Yosemite National Park (37° 47' 37.32" N, 119° 51' 09.42" W; 1420 m elevation). Given the size of these trees (mean dbh \pm SEM = 121.9 \pm 4.3 cm, max. = 223.1 cm), the height to the base of the crown was often > 15 m, and as such crown scorch was only observed on one tree. However, the lower boles of all trees were heavily charred by fire, and bark consumption was evident on some trees. We applied 100.0 g of SPLAT® Verb to unbaited trees as four 25.0-g dollops (see Experiment 3 for complete method). Of the 86 trees that were treated in 2014, none were colonized by *D. ponderosae* that year. These trees were not retreated in 2015, and therefore left unprotected. Many of these trees and nearby trees that had never been treated with SPLAT® Verb were observed being colonized by *D. ponderosae* in May of 2015.

As with other formulations of verbenone, it is possible that animals could contact and/or consume dollops of SPLAT® Verb. For example, while never observed directly, in Experiment 1, we found evidence of small claw marks on several dollops on *P. contorta* that we attributed to contact by red squirrels, *Tamiasciurus hudsonicus* (Erxleben). Syracuse Environmental Research Associates (2000) conducted a risk assessment of verbenone, and concluded it was unlikely that consumption by wildlife would have a detectable impact on any species. While associated toxicology data are scarce, acute oral LD₅₀ values for rats, *Rattus* spp., the only mammal studied, are estimated at 1,800 mg/kg for females and 3,400 mg/kg for males (Syracuse Environmental Research Associates 2000). Verbenone administered to bobwhite quail, *Colinus virginianus* (L.), at doses of 39–300 mg/kg in corn oil had no effect on behavior or health (Syracuse Environmental Research Associates (2000). Verbenone has mixed effects on several species of insects (e.g., Lindgren and Miller 2002), however a common predator of bark beetles in western North America, *Temnochila chlorodia* (Mannerheim) (Coleoptera: Trogossitidae), is attracted to (–)-verbenone, and its impact on bark beetles may therefore be enhanced by treatments containing verbenone (Fettig *et al.* 2007). The inert ingredients of SPLAT® Verb have been certified as food safe by the USEPA (Mafra-Neto *et al.* 2013), and SPLAT® Verb has been granted organic production status by the United States Department of Agriculture.

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This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides in the United States must be registered by appropriate state and/or federal agencies before they can be recommended. This article was written and prepared by US Government employees on official time, and is, therefore, in the public domain and not subject to copyright.

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History of the balsam woolly adelgid, *Adelges piceae* (Ratzeburg), in British Columbia, with notes on a recent range expansion

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ABSTRACT

The balsam woolly adelgid, *Adelges piceae* (Hemiptera: Adelgidae), was introduced from Europe into eastern North America around 1900 and independently into western North America sometime before 1928. It was first detected causing damage in North Vancouver, British Columbia, in 1958. Since then, it has slowly spread to adjacent areas of southwestern B.C. Surveys from 2011 to 2013 confirmed the presence of *A. piceae* in the Cascades Forest District and in the town of Rossland, B.C., which are outside the pre-2014 quarantine area. Until these recent detections, provincial quarantine regulations have been the principle tool employed to prevent anthropogenic spread of the adelgid through the restriction of movement of potentially infested seedlings and nursery stock from infested coastal regions of British Columbia into the highly susceptible high-elevation *Abies lasiocarpa* stands in the Interior forests. We provide a historical overview of the quarantine regulations enacted since 1966, review the distribution of *Adelges piceae* since the first confirmed records of establishment as documented by historical survey records, and document the extent of recent survey efforts and new detections in interior subalpine fir forests.

INTRODUCTION

The balsam woolly adelgid, *Adelges piceae* (Ratzeburg) (Hemiptera: Adelgidae), occurs on both coasts of North America (NA) and can cause extensive tree damage and mortality to native *Abies* species. It was introduced into eastern NA from Europe before 1900 (Foottit and Mackauer 1980) and independently into western North America (Hain 1988), where it was first reported near San Francisco in 1928 (Annand 1928). *Adelges piceae* was first reported from British Columbia (B.C.) by E. P. Venables and R. Hopping (Anon. 1938). Those reports noted its detection, along with *Adelges nüsslini* (Börner), on *Abies procera* Rehder (= *A. nobilis* (Douglas ex D. Don) Lindley) in Vancouver. In 1958, *A. piceae* was discovered damaging a Pacific silver fir, *Abies amabilis* (Douglas ex Loud.) Dougl. ex J. Forbes, planted as an ornamental in North Vancouver, B.C. (Silver 1959). Surveys to delimit the range of this introduced pest in the province documented in the Canadian Forest Invasive Alien Species (CanFIAS)⁶ database (1958–1998; Nealis *et al.* 2015) quickly demonstrated its presence on native and ornamental firs in drainages near Vancouver, as well as on southern Vancouver Island. In 1959, it was found attacking grand fir (*Abies grandis* (Douglas ex D. Don) Lindl.) at Thetis Lake, near Victoria, and

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the following year, it was detected at a commercial nursery near Victoria on white fir (*Abies concolor* (Gord. & Glend.)) imported from Holland five years prior to the detection.

The degree of susceptibility to damage and mortality varies amongst the three native *Abies* species in B.C.: damage is generally moderate in grand fir, while that in both Pacific silver fir and subalpine fir (*A. lasiocarpa* (Hook.) Nutt.) is more severe. Subalpine fir is the most susceptible of the true firs in the Pacific Northwest (Mitchell 1966; Hain 1988).

In North America, *A. piceae* is anholocyclic on the ancestral secondary host, *Abies* (Havill and Footitt 2007). There are two or more generations per year (Mitchell *et al.* 1961). The life stages consist of eggs, three nymphal instars, and the adults. Eggs hatch into crawlers, which are the only stage capable of independent movement and dispersal (Balch 1952). Crawlers select feeding sites, settling either on the bark of the main stem and larger branches or at the base of vegetative or reproductive buds, then insert their stylets into the cortical parenchyma. Once the stylets are inserted, the insect remains sessile for its life. As the adelgids develop, they molt and secrete waxy threads that appear as dense white, woolly, or ‘cottony’ masses. Adult females lay their eggs within the woolly masses—each of which may contain more than 200 amber-coloured eggs (Balch 1952).

Feeding by *A. piceae* on twigs produces gouting at nodes and inhibits new growth, reducing tree vigor. Stem infestations are a more severe form of attack by *A. piceae*. The tree responds to adelgid feeding by producing a type of compression wood in the sapwood called “rotholz”. This abnormal growth of the sapwood tissue inhibits water flow within the tree and eventually leads to tree death (Balch 1952; Livingston *et al.* 2000). The presence of rotholz in the annual rings of attacked trees in North Vancouver suggested that *A. piceae* had been present in southwestern B.C. for 8–11 years before its discovery (Silver 1959).

In British Columbia, *A. piceae* has historically been restricted primarily to southwestern B.C., with the Coast and Cascade Mountains acting as natural barriers to eastward range expansion into Interior stands of *Abies lasiocarpa* growing at high elevations. To protect the susceptible subalpine fir stands in the interior of the province, the Province of British Columbia has maintained regulatory restrictions on the production and movement of all living *Abies* spp., as well as logs and cut Christmas trees since 1966. Coulson and Witter (1984) observed that the initial quarantine restrictions implemented against *A. piceae* in the mid-1960s were effective in stabilizing the infestation boundary after 1967. In contrast, in the absence of any regulatory restrictions in the western United States, *A. piceae* has expanded its range extensively through Washington, Idaho and Montana. It is now present in all U.S. counties bordering B.C. (Hayes 2015; Liebhold *et al.* 2015) and has caused extensive mortality of *A. lasiocarpa* in the Sawtooth National Forest in southern Idaho (Livingston *et al.* 2000; Livingston and Pederson 2010).

In 2008, a single branch sample was submitted from a 60-year-old *Abies lasiocarpa* planted as an ornamental at low elevation near Rossland, B.C. The condition of the sample precluded a definitive identification of the pest. In 2009, symptoms of branch attack by *A. piceae* were reported from the Cascades Forest District immediately east of the pre-2014 quarantine zone. These reports prompted additional surveillance for *A. piceae* to determine the current extent of its range in B.C. Initial surveillance efforts focused on detection of host trees with visible symptoms of attack, such as gouting or the presence of white woolly masses associated with heavy stem attack. This study reviews the historical records of *A. piceae* detections in B.C., the history of provincial regulations to prevent anthropogenic dispersal of the adelgid to uninfested regions of the province, and reports new locations in the interior of B.C. where it is now established.

METHODS

Historical surveys and collections of *Adelges piceae* in B.C. Historical collections of *A. piceae* documented by the Canadian Forest Service Forest Insect and Disease Survey (FIDS), Pacific Forestry Centre, Victoria, B.C., and records of detections extracted from both published and unpublished file reports compiled in the CanFIAS database by Nealis *et al.* (2015) were retrieved and combined with locality records for positive and negative collections of the pest documented in this study to provide an overview of both the species' occurrence in B.C., and the areas surveyed at which *A. piceae* was not detected. Scatter plots of the positive and negative collections are also presented to visualize the pattern of spread of the pest in coastal B.C. between 1957 and 1995.

In addition to latitude and longitude, the CanFIAS database provides an estimate of the spatial accuracy of the locality information for all positive and negative collection records for both point source collections made by FIDS between 1957 and 1995 and for records generated from annual survey reports of FIDS, provincial aerial survey reports, and other miscellaneous reports. The spatial accuracy of CanFIAS records for *A. piceae* extracted from the aforementioned reports were compared to those generated during individual collection events documented by the original collection records to estimate the spatial accuracy of records derived from both types of records.

History of *Adelges piceae* Regulation in B.C. Copies of the text of all regulations enacted under the *Plant Health Act* of B.C. pertaining to *A. piceae* were obtained from the Legislative Library at the Provincial Legislature in Victoria, B.C. Titles of each regulation and date of enactment, along with comments on the purpose of the Order in Council (O.I.C.) or changes in the areas regulated, are summarized in Table 1. Maps of the areas regulated for *A. piceae* were developed from the descriptions of the areas regulated by each O.I.C. to illustrate the extent of the area regulated in each change. Significant changes to the regulations documented in the applicable O.I.C.'s were summarized.

2011–2014 Surveillance for *Adelges piceae*. In July and September 2011, *Abies* spp. branches exhibiting symptoms of attack by *A. piceae* were sampled with pole or hand pruners. Samples were collected from the lower one-third of the crown of trees that showed evidence of gouting or tree decline in the Coquihalla Summit Recreation Area. Branch samples were returned to the laboratory and held with the cut ends in water in buckets at room temperature for approximately 10 days to induce adelgid development and production of white woolly flocculence. Branch samples were inspected under a 10X magnifying stereomicroscope for evidence of *A. piceae* life stages. In October 2013, two lower branches of both mature trees and advanced regeneration of subalpine fir growing in the vicinity of Rossland, B.C., were sampled by hand. Samples from individual trees were bagged separately. Branches were examined under a stereomicroscope within 7 days of collection, and all adelgid life stages recovered were preserved in 95% ethanol. A subsequent survey was done in the Rossland area and at high-elevation sites across the southern interior of B.C. in 2014 to assess the extent of *A. piceae* establishment.

Adelgid samples were forwarded to RGF at the Canadian National Collection, Agriculture and Agri-Food Canada, Ottawa, for identification. Species identifications were based on an examination of species morphology, using slide-mounted specimens and sequencing of the barcode region of the mitochondrial gene Cytochrome C oxidase subunit I (COI). DNA was extracted from each of the submitted samples, and COI was amplified, sequenced and compared to a reference library of adelgid sequences (Footitt *et al.* 2009). COI sequences for all samples positive for *A. piceae* were deposited in GenBank, National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda MD, U.S.A. <http://www.ncbi.nlm.nih.gov/genbank/>.

Table 1
Chronological record of significant detections of *Adelges piceae* in British Columbia, 1937–1998.

Year	Location	<i>Abies</i> Host ¹	Reference ²	Comments
1937	Vancouver, B.C.	<i>procera</i>	Anon. 1938	First record of detection in B.C. <i>Adelges nusslini</i> (Börner) also present on <i>A. procera</i>
1958	North Vancouver, B.C.	<i>amabilis</i>	Silver 1959	First record of establishment and damage
1959	Thetis Lake near Victoria, B.C.	<i>grandis</i>	CFS FIDS	First record on Vancouver Island
1959-1960	Mountains near North and West Vancouver; west shore of Howe Sound. Fig. 3a	<i>amabilis</i> .	CFS FIDS	<i>Adelges piceae</i> confirmed to be widespread near Vancouver across Howe Sound
1960	Near Victoria, B.C.	<i>concolor</i>	CFS FIDS record	Commercial nursery
1961-1966	Mainland NE to Jervis Inlet and east to Alouette Lake; west of Saanich Inlet north to Duncan, southern Vancouver Island, Fig. 3b.	<i>amabilis</i> ; <i>grandis</i>	Ruppel and Allen (1964, 1965); CFS FIDS	Mainland expansion east and north-east of previous finds; first records on <i>A. amabilis</i> on Vancouver Island
1967	Mainland east and west shores of Harrison Lake from Agassiz, northwards, Fig. 3c.	<i>lasiocarpa</i>	CFS FIDS	Mainland expansion north up the Harrison Lake drainage
1967	Near Sooke, Nanaimo and Gordon River on Vancouver Island, Fig. 3c.	<i>amabilis</i>	Alexander 1967	Expansion beyond east coast of Vancouver Island
1967	Southern Okanagan near Oliver and Penticton	<i>alba</i> , <i>concolor</i>	CFS FIDS; Wood 1968; Wood <i>et al.</i> 1968	Heavily infested ornamental <i>A. alba</i> near Oliver, infested ornamental <i>A. concolor</i> in Penticton.
1970-1979	No significant range expansion identified on Vancouver Island or the mainland			All newly discovered populations within the Quarantine Zone boundaries
1986	Near Powell River on the mainland	Not specified	CFS FIDS	Record from provincial Forest Service collection submitted for identification

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Year	Location	<i>Abies</i> Host ¹	Reference ²	Comments
1987	West Thurlow Island east of Sayward, Fig. 3e	<i>amabilis</i>	CFS FIDS	Infested trees at 10 locations north of 1977 Quarantine Zone boundary
1989	China Creek near Port Alberni, Vancouver Island, Fig. 3e	<i>amabilis</i>	Humphreys and Clarke 1990	2 ha stand near Quarantine Zone boundary
1993	Chute Creek near Campbell River; Nahmint Lake near Port Alberni, Fig. 3f	<i>amabilis</i> ; <i>grandis</i>	CFS FIDS	Gouting and adelgids in foliage samples; in grand fir plantation. Beyond 1992 Quarantine Zone boundary
1994	Mainland east of Lillooet Lk.; Birkenhead River area, Fig. 3f	<i>amabilis</i> ; <i>lasiocarpa</i>	CFS FIDS; Turnquist <i>et al.</i> 1995	In mature stands beyond the 1992 Quarantine Zone boundary
1995	Infested mature stands on central Vancouver Island at five locations; grand fir at Menzies Bay near Campbell River; Anderson River east of the Fraser River, Fig. 3f	<i>amabilis</i> ; <i>grandis</i>	CFS FIDS; Turnquist and Humphreys 1996	Expansion northward on Vancouver Island beyond 1992 Quarantine Zone boundary; first record east of the Fraser River
1998	Davis Bay on Texada Island, Fig. 3f	<i>amabilis</i>	CFS FIDS	Record from provincial Forest Service collection

¹ *alba* = *Abies alba* Mill.; *amabalis* = *Abies amabilis* (Douglas ex Loud.) Dougl. ex J. Forbes; *concolor* = *Abies concolor* (Gord. & Glend.); *grandis* = *Abies grandis* (Douglas ex D. Don) Lindl.); *lasiocarpa* = *Abies lasiocarpa* (Hook.) Nutt.); *procera* = *Abies procera* Rehder

² CFS FIDS = original collection records of the Forest Insect and Disease Survey, Canadian Forest Service, Victoria, B.C.; available in the CanFIAS database (Nealis *et al.* 2015)

RESULTS

Historical surveys and collections of *Adelges piceae* in B.C. The original dataset extracted from the CanFIAS database on 2 Sept. 2015 contained 480 positive records and 1318 negative records (i.e., records of collections made specifically to detect the adelgid in which it was not found) for *A. piceae*. Two hundred and twelve of the positive records documented actual collection events with the remaining 268 records being derived from published and unpublished reports. The mean (\pm standard deviation) and maximum spatial accuracy of records derived from collection events were 6.18 ± 2.1 km and 8.8 km, respectively, while the mean and maximum spatial accuracy of records derived from literature were 67.9 ± 61.8 km and 219.5 km, respectively. Sixty-five of the positive records extracted from reports or literature exceeded the maximum spatial accuracy for collection events (8.8 km) and all but one were excluded from mapping. The single literature record that exceeded the spatial accuracy limit by 1.08 km was found to represent a valid collection made on urban ornamentals in Penticton in 1967 (Wood 1968) and was retained. The final dataset of positive records from CanFIAS consisted of the 212 collection events and 205 records generated from the literature. These occurrence records, as well as the locations of positive collections obtained in this study, are mapped in Fig. 1. Similarly, 47 literature records that exceeded the spatial accuracy limit and one literature record based on damage only included in the negative collection records for *A. piceae* extracted from the CanFIAS database were excluded from mapping. In total, 1270 CanFIAS negative records were mapped along with the 10 negative collection records derived from this study to document the areas surveyed for *A. piceae* at which the pest was not detected (Fig. 2).

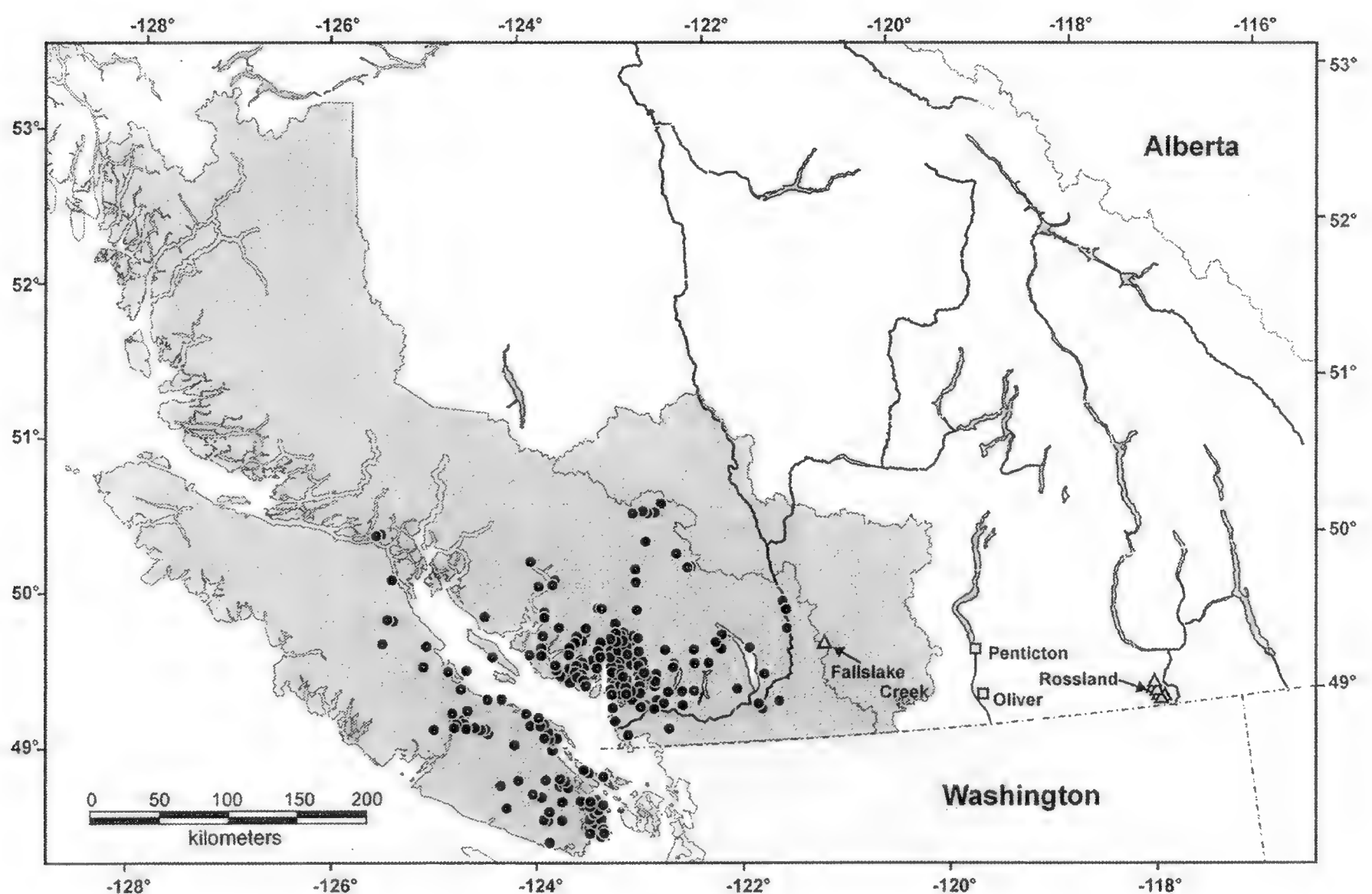


Figure 1. Location of positive detections of *Adelges piceae* in British Columbia between 1958 and 2014; locations denoted by red circles denote positive collection records extracted from the CanFIAS database (Nealis *et al.* 2015); locations denoted by red squares are derived from this study (see Table 2). The two collections denoted by red triangles represent detections on urban ornamentals that were eradicated.

We also mapped the positive and negative detections of *A. piceae* generated from point source collection data pooled with records with equivalent spatial accuracy derived from survey reports and literature from 1958–1998 to illustrate the spread of *A. piceae* in coastal B.C. (see Fig. 3). The importance of the latter data sources in documenting spread is illustrated in Fig. 3b. Records to the northwest of the dashed line on the mainland represent areas infested by the adelgid, as determined by surveys records documented in unpublished reports (Ruppel and Allen 1964, 1965) that were not reported in collection records. A chronology of significant range expansions of the adelgid in coastal B.C. are summarized in Table 1.

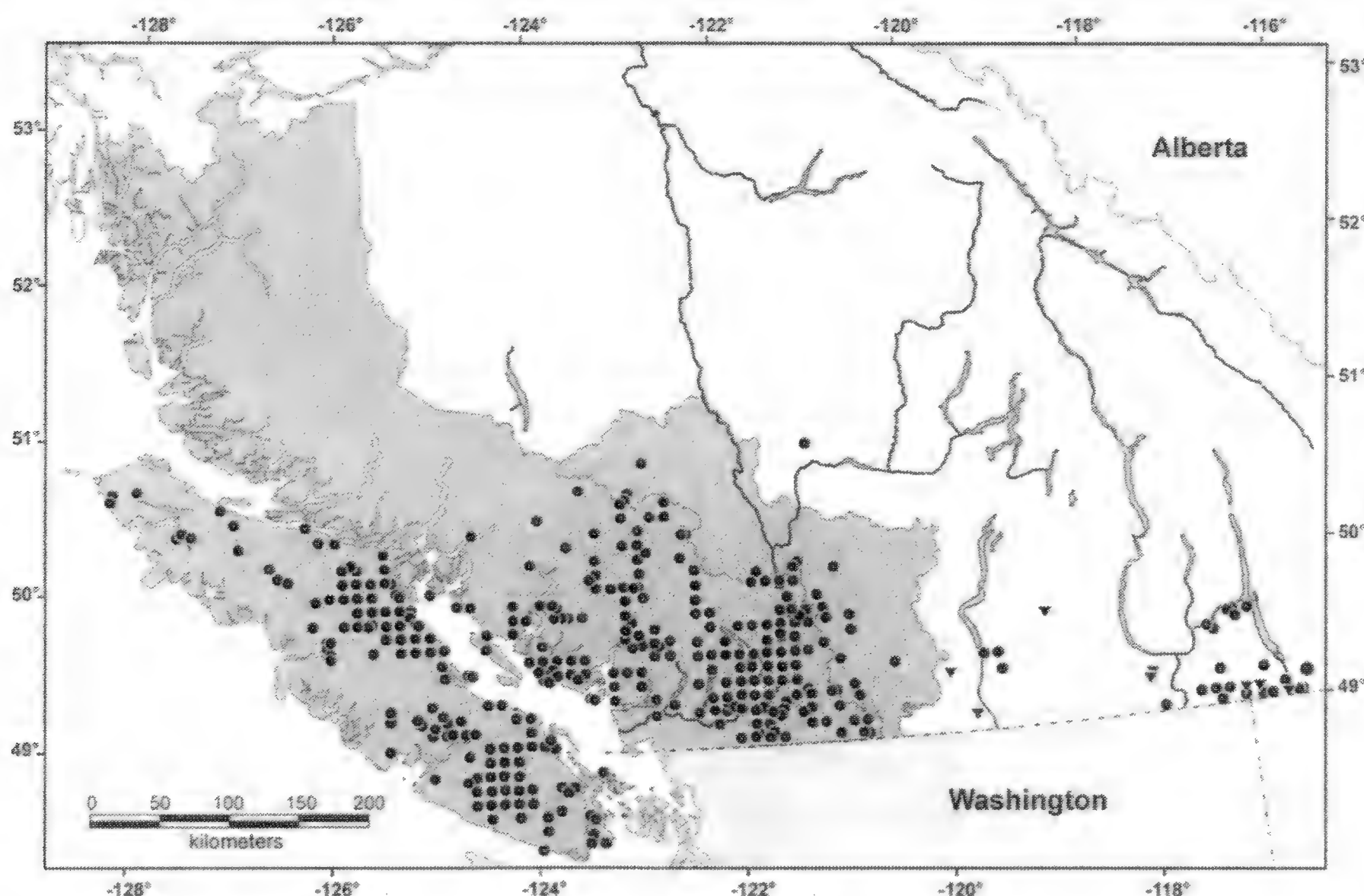


Figure 2. Locations surveyed for *Adelges piceae* in British Columbia between 1958 and 2014, at which the pest was not detected; locations denoted by unfilled circles denote locations negative collection records extracted from the CanFIAS database (Nealis *et al.* 2015); locations denoted by unfilled squares are derived from this study (see Table 3).

In the two years following the discovery of *A. piceae* in North Vancouver, populations were detected in the mountains north of Vancouver and across Howe Sound, as well as at a few localities near Victoria on Vancouver Island (Table 1; Fig. 3a). From 1959–1964, infestations were discovered on the mainland as far northwest as Jervis Inlet, on the Sunshine Coast, and as far east as Alouette Lake, in the Fraser Valley. On Vancouver Island, expansion was limited to a few additional populations discovered on the west shore of Saanich Inlet (Table 1; Fig. 3b). By 1969, additional infested stands had been discovered on the east coast and interior of Vancouver Island as far north as Nanaimo, and a significant northward expansion of the infested area in the Harrison Lake drainage had been found on the mainland (Table 1; Fig. 3c). Between 1970 and 1979, the range of *A. piceae* expanded slightly eastward on the mainland, and a further expansion to the north within the quarantine zone was detected on Vancouver Island (Table 1; Fig. 3d). By 1989, the adelgid had been found in stands near the quarantine zone boundary on Vancouver Island and near Powell River on the mainland. However, the most significant event during the decade was the detection of mature stands of *A. amabilis* infested by the adelgid on West Thurlow Island (WTI), between northern Vancouver Island and the adjacent mainland coast, 100 km and 140 northwest of previously detected infestations on the mainland and Vancouver Island, respectively (Table 1; Fig. 3e). The last

collections of *A. piceae* documented in the CanFIAS database were made between 1990 and 1998. Expanded surveys to delimit the range of the adelgid on northern Vancouver Island after the detection of infestations on WTI (Fig. 3e) led to the detection of *A. piceae* as far north of Campbell River (Table 1; Fig. 3f). On the mainland, expansion of *A. piceae* to both the north and east was detected in the Fraser River drainage, with the adelgid being found for the first time east of the Fraser River. The adelgid had also expanded northward along drainages emptying into the Squamish River and was present beyond the 1992 regulated area at multiple locations in the Birkenhead Lake area (Table 1; Fig. 3f). After the completion of the 1995 survey year, organized surveys for forest pests were no longer conducted by the Canadian Forest Service. In subsequent years, surveys for *A. piceae* were conducted by provincial forestry staff. The single record from 1998 in the CanFIAS database was submitted by provincial forest service staff and documents the presence of *A. piceae* on Texada Island in the Strait of Georgia, south of Powell River (Fig. 3f).

History of *Adelges piceae* Regulation in B.C. By 1965, *A. piceae* was recognized as a serious potential threat to *Abies* in B.C., and steps were taken to manage its impact through establishment of quarantine regulations, supported by surveys of infestation boundaries and damage, a ban on movement of logs from infested areas during periods when the adelgid was actively reproducing, and expansion of research programs (Vyse 1971). These activities were cost-shared between the federal and provincial governments and initially were enabled provincially through enactment of Order in Council (O.I.C.) 1137 (14 Apr. 1966). They were completed the following year under a second agreement (O.I.C. 2363, 25 July 1967; Table 2). The collaborative surveys enabled by these O.I.C.'s led to the discovery of limited northwards range expansion on southern Vancouver Island and no range expansion on the Lower Mainland. A benefit of these surveys was the first extensive documentation of stands in the Lower Mainland and on Vancouver Island that were free of *A. piceae* infestations (Table 1; Fig. 3d). Additionally, these extensive surveys led to the discovery of *A. piceae* in Penticton, B.C.

Adelges piceae was first regulated under the *Plant Protection Act* (chapter 287 of the *Revised Statutes of British Columbia, 1960*) with the approval of *B.C. Reg. 58/66* (Table 2). The regulation prohibited the shipment or transport of any living *Abies* spp., as well as the production of all *Abies* species for commercial purposes, eliminating the production of *Abies* seedlings for reforestation, as well as the commercial production of *Abies* species grown as ornamentals or Christmas trees. The initial regulation applied province-wide to protect the highly susceptible *A. lasiocarpa* in the high-elevation Interior forests of B.C. and slow the spread of the insect (Wood 1968). *Abies* seedling stocks at forest nurseries were destroyed and operational planting of all *Abies* ceased with the imposition of the 1966 regulation (Vyse 1971), even though species of *Abies* such as Pacific silver fir (*A. amabilis*) were desirable for silviculture at mid-elevations in coastal forests where other conifers frequently failed to establish (Carrow 1973).

In 1977, the provisions of *B.C. Reg. 58/66* were rescinded by O.I.C. 44 and replaced by *B.C. Reg. 7/77*, the *British Columbia Balsam Woolly Aphid Regulations, 1976* (Table 2). Annual permits were required to grow and sell *Abies* provincially and the first regulated area, based on known infestations in Ranger Districts (Fig. 4a), was established. Movement of all *Abies* species grown within the regulated area to areas beyond its boundaries was prohibited, as was movement of cut trees or foliage of *Abies* spp. between January 31 and November 1. The latter regulation allowed for the movement of cut Christmas trees and foliage after research demonstrated that the adelgid did not survive on cut trees (Woods 1967).

New finds of the adelgid beyond the boundaries of the area regulated by *B.C. Reg. 7/77* (Table 1) led to an expansion of the regulated area (Table 2; Fig. 4b) with the adoption of *B.C. Reg. 414/92* established by O.I.C. 1604, approved on October 22, 1992 (Turnquist and Harris 1993). Movement of trees (living trees with roots, including both seedlings and those produced by tissue culture) from inside to outside of quarantine area

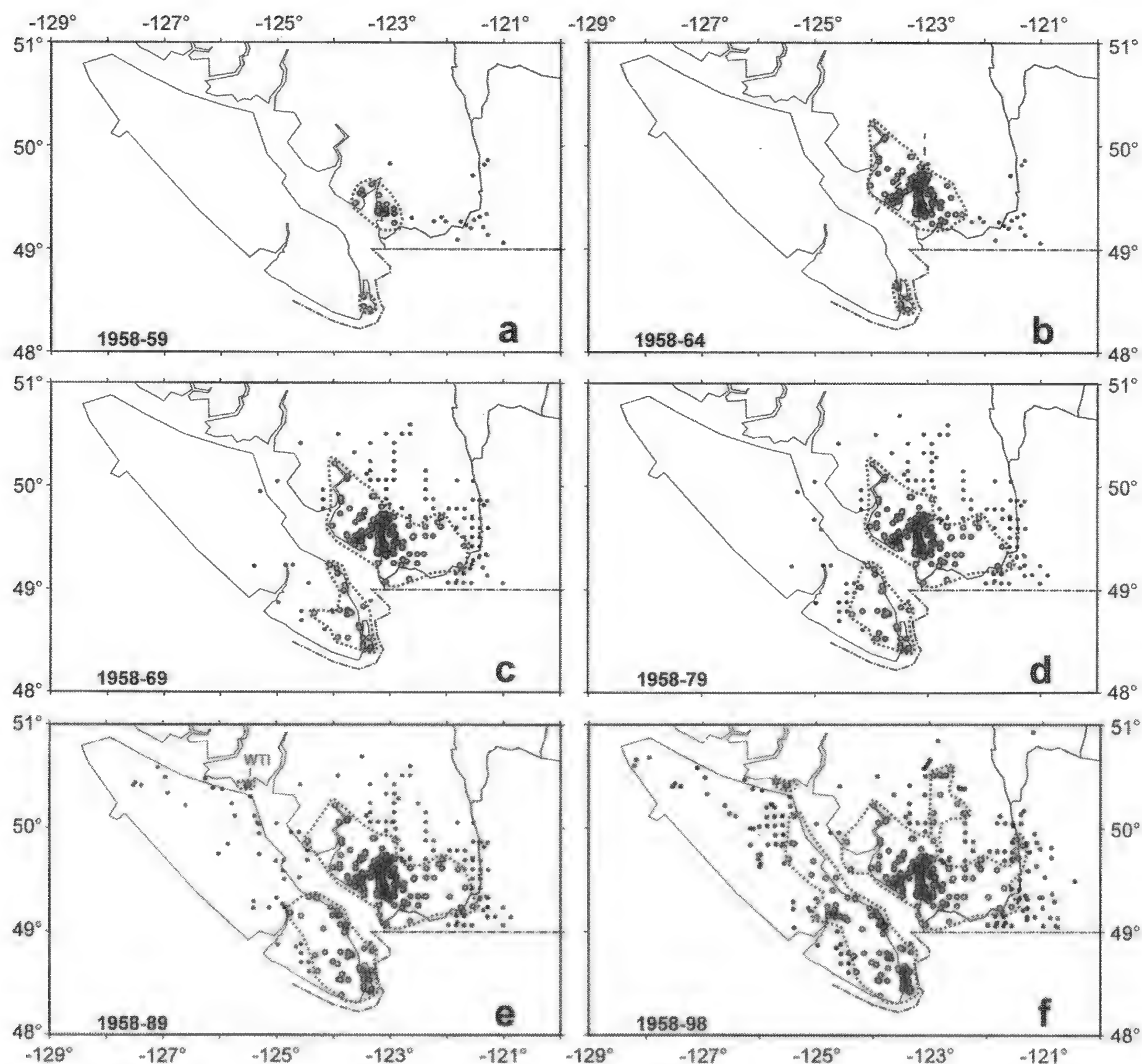


Figure 3. Expansion of *Adelges piceae* in coastal British Columbia between 1957 and 1995, as documented by positive and negative collection records (denoted in red and black, respectively), extracted from the CanFIAS database (Nealis *et al.* 2015). Islands between Vancouver Island and the mainland have been omitted for clarity. Red dashed lines delimit areas in which *A. piceae* has been detected: **a.** collections recorded in 1958 and 1959; **b.** cumulative collections between 1958 and 1964. Positive records to the northwest of the dashed line on the mainland are derived from aerial survey records documented in Ruppel and Allen (1964, 1965). Note significant expansion of the area infested to the southeast and northwest; **c.** cumulative collections between 1958 and 1969. Expansion to the northwest is evident on southern Vancouver Island and to the northeast on the mainland; **d.** cumulative records between 1958 and 1979; **e.** cumulative records between 1958 and 1989. Populations on the mainland and on Vancouver Island expanded to the northwest, and a satellite population was discovered on West Thurlow Island (WTI); and **f.** cumulative records from 1958 to 1998. Between 1990 and 1995, survey efforts documented significant northward expansion of *A. piceae* in the Fraser, Lillooet and Squamish river drainages on the mainland, as well as expansion to the northwest along the east coast of Vancouver Island to Campbell River.

boundaries continued to be prohibited; however, the modified regulations allowed the movement of logs of *Abies* spp. out of a quarantine zone if the logs were transported and stored in water and promptly processed as research had found that they posed minimal risk for dispersal of the adelgid (Atkins and Woods 1968). The sale of cut trees or foliage continued to be prohibited in B.C. between January 31 and November 1.

In 2000, a minor change was made to the *Balsam Woolly Adelgid Regulation* (Table 2). *B.C. Reg. 169/2000*, enabled by O.I.C. 726/2000, eliminated the need for the tagging of trees offered for commercial sale to identify that they had been grown under permit.

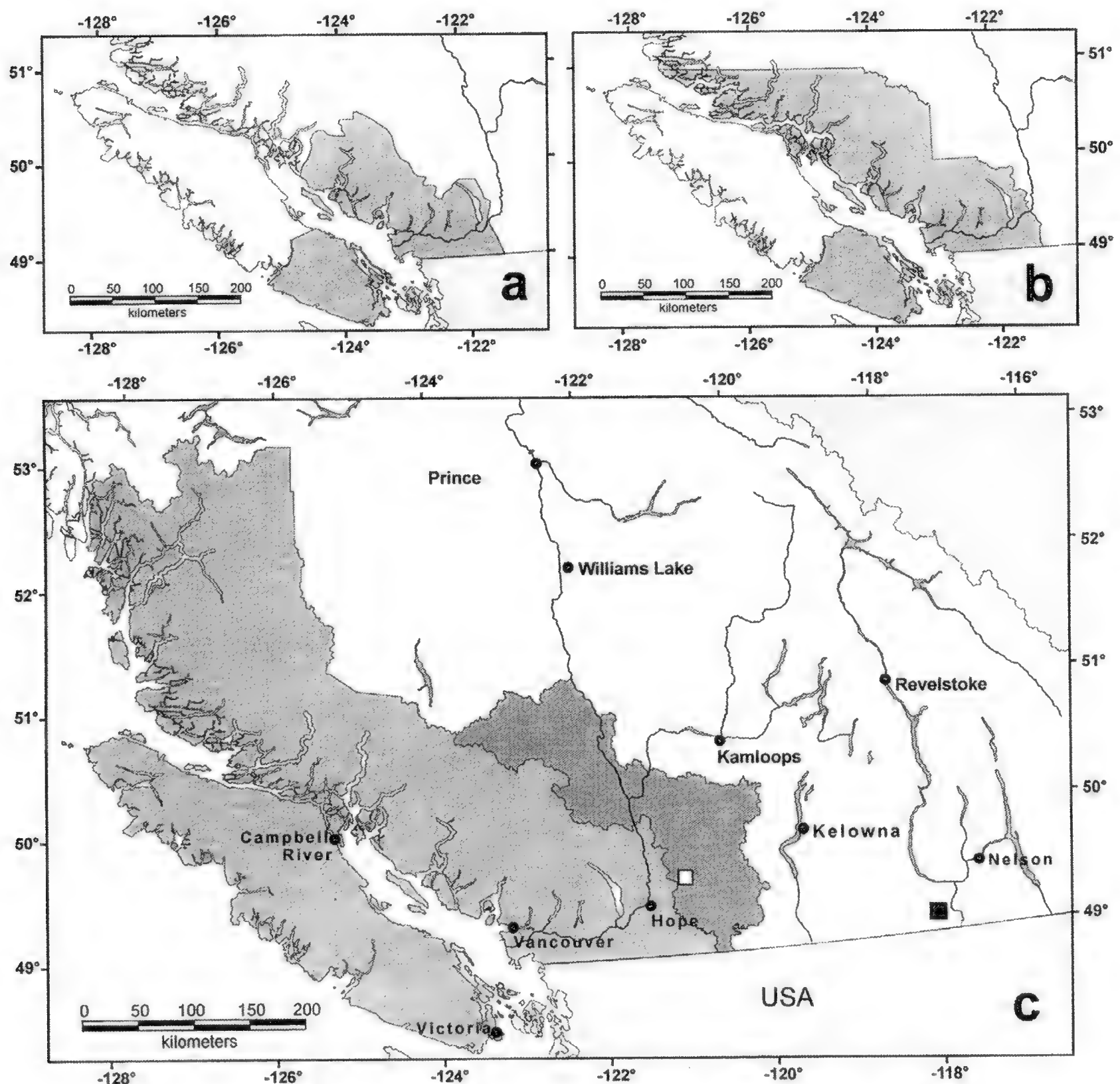


Figure 4. Historical change in areas of southwestern British Columbia regulated for *Adelges piceae*: **a.** area regulated in 1977. As no official record of the boundaries of the “Ranger Districts” named in Order in Council (O.I.C.) 1977-44 was retained, the area regulated on the mainland was approximated from other sources; **b.** area regulated by O.I.C. 1992-1604; and **c.** area regulated by O.I.C. 2006-493 (light grey) and the area added by O.I.C. 2014-361 (dark grey). The locations of the *Adelges piceae* detections at Falls Creek and Rossland are designated by unfilled and filled black squares, respectively.

With the detection of new finds east of the known distribution (Table 1), the *Balsam Woolly Aphid Regulation* was amended by *B.C. Reg. 213/2006*, established by O.I.C. 493/2006. The definition of “log” was changed to clarify that branches were not permitted on logs, and the definition of “quarantine zone” was repealed and replaced by “quarantine area” (described in the regulation’s appendix). The only substantive change to the regulation was that Appendix A of *B.C. Reg. 414/92* was repealed and an expanded quarantine area defined in the Appendix to *B.C. Reg. 213/2006* was created (Table 2; Fig.

4c). The most recent amendment to the *Balsam Woolly Adelgid Regulation, B.C. Reg. 137/2014*, of June 30, 2014, added the Cascade Forest District to the quarantine area after recent surveillance indicated establishment of the adelgid beyond the boundaries of the 2006 regulated area (Table 2; Figure 4d).

Table 2
Summary of the provincial Orders in Council and regulations arising from each Order in Council (O.I.C.) enacted against *Adelges piceae*. Changes to the area regulated for *Adelges piceae* by specific O.I.C. are noted in the comments.

Order in Council ¹			Regulation	Name	Comments
Year	Number	Enactment Date			
1966	460	16 Feb. 1966	<i>B.C. Reg. 58/66</i>		All of British Columbia
1966	1137	14 Apr. 1966			Cost sharing agreement for surveys and acquisition of contaminated nursery stock
1967	2363	25 Jul. 1967			Cost sharing agreement for surveys and acquisition of contaminated nursery stock
1977	44	7 Jan. 1977	<i>B.C. Reg. 7/77</i>	<i>British Columbia Balsam Woolly Aphid Regulations, 1976</i>	All portions of 10 mainland and Vancouver Island Ranger Districts and the infested portions of two Ranger district in Vancouver Region (see Fig 3a)
1992	1604	22 Oct. 1992	<i>B.C. Reg. 414/92²</i>	<i>Balsam Woolly Adelgid Regulation</i>	
2000	726	18 May 2000	<i>B.C. Reg. 169/2000²</i>	<i>Balsam Woolly Adelgid Regulation</i>	
2006	493	13 Jul. 2006	<i>B.C. Reg. 213/2006²</i>	<i>Balsam Woolly Adelgid Regulation</i>	South Coast Forest Region and West Coast Forest Region
2014	361	5 Jun. 2014	<i>B.C. Reg. 137/2014²</i>	<i>Balsam Woolly Adelgid Regulation</i>	Cascades Forest District; South Coast Forest Region and West Coast Forest Region

¹ Text of all Orders in Council for British Columbia, including amendments up to *B.C. Reg. 137/204*, June 30 2014, pertaining to *Adelges piceae* were accessed at the following URL: http://www.bclaws.ca/Recon/document/ID/freeside/11_414_92 (accessed 10 October 2015).
² Amendment to *B.C. Reg. 414/92*

2011–2014 Surveillance for *Adelges piceae*. Locations sampled between 2011 and 2014 at which the presence of *Adelges piceae* was confirmed are documented in Table 3 and mapped in Figure 1; locations negative for the presence of *A. piceae* are presented in Table 4 and mapped in Figure 2. The presence of the adelgid in the Coquihalla Summit Recreation Area in the Cascades Forest District is not surprising, as the adelgid was known to be present in high-elevation coastal forests to the west of the detection. The Cascade Forest District was added to the quarantine area in June 2014 as a result of the surveys conducted. However, the presence of established populations of *A. piceae* in the Southern Interior of the province around Rossland (Table 2; Fig. 1), more than 200 km east of known infestations in B.C., was unexpected.

DISCUSSION

The discovery of *A. piceae* at multiple locations near Rossland is the first detection of this pest in the subalpine-fir forests in the interior of the province. Infested stands were detected in this study up to 9.5 km north and 3.8 km south of Rossland. Although *A. piceae* was detected in the southern Okanagan in 1967, the incursions were restricted to a few non-native urban ornamentals (*A. alba* and *A. concolor*) in Oliver and Penticton, and the populations were subsequently eradicated. Surveys of subalpine fir from Penticton south to the U.S. border provided no evidence that *A. piceae* had dispersed from the two low-elevation urban infestations into natural stands (Wood 1968; Wood *et al.* 1968). Symptoms of attack were not evident at the majority of the locations surveyed for *A. piceae* in the Rossland area. Severe gouting, stem attack and limited mortality were only evident at the golf course in Rossland, the lowest-elevation site sampled. Gouting was also documented on subalpine fir at the Highway 3B location (Table 2). At all other locations sampled, gouting was not evident, although a slight thickening of some nodes was noted at two other locations (Table 3; LH, personal observations).

It is unlikely that the *A. lasiocarpa* growing at the Rossland golf course was infested with *A. piceae* when planted some 60 years ago, as subalpine fir is highly susceptible to infestations and generally succumbs rapidly after infestation. Those subalpine firs that succumb first after infestation by *A. piceae* are often growing on the best sites (stream bottoms, benches and around meadows), with the severest damage occurring at the lowest elevations starting at around 915 meters (3000 ft.; Mitchell 1966), a description that closely resembles the golf course site. The source of this infestation could not be determined with any certainty; however, it is suspected that it has arisen from aerial dispersal of crawlers from nearby counties in Washington or Idaho, where populations of *A. piceae* have been documented (Liebhold *et al.* 2015). Alternatively, because asymptomatic *A. lasiocarpa* infested with *A. piceae* were easily found within Rossland and adjacent forested areas, the possibility remains that the adelgid may have originally been introduced on ornamental firs transported from other infested regions of the province. Unlike earlier detections of *A. piceae* on non-native urban ornamentals in the southern Okanagan (European silver fir, *Abies alba* Mill. and white fir, *A. concolor*; Wood 1968; Wood *et al.* 1968), plantings of non-native firs were not observed during surveys in Rossland. In addition, the latter scenario is improbable, as quarantine regulations have restricted the movement of ornamental *Abies* spp. from infested areas of the province to uninfested areas since 1966. While quarantine restrictions were also in place in 1967, the infested *A. alba* detected at Oliver were imported from Europe 29 years prior to the discovery of the adelgid. Neither the origin nor date of planting for the two white firs in Penticton was determined, although the trees were of similar height and diameter at breast height to the silver firs in Oliver, suggesting that they also were planted prior to the introduction of any quarantine regulations. Neither of these ornamental firs were killed by *A. piceae*, and gouting is not expressed on either species in the Pacific Northwest (Mitchell 1966).

Table 3
Collection data, observations on tree condition, and GenBank Accession numbers for partial COI sequences from samples positive for the presence of *Adelges piceae* documented in this study.

Collection Location	Lat., Long. (Dec. Deg.)	Year	Elev. (m)	Comments	GenBank Accession No.
Falls Creek, Coquihalla Summit Recreation Area	49.6101, -121.0559	2011	1210	Tree decline, gouting, numerous adelgids in buds, on <i>Abies amabilis</i>	KJ445727
Rossland, Redstone Golf Course	49.0677, -117.7844	2013	825	Heavy stem and branch attack by adelgids, gouting, tree decline and mortality on 60 year old <i>A. lasiocarpa</i> planted on the golf course	KJ445729
Rossland, junction of Plewman Way and Spokane St.	49.0836, -117.8016	2013	1100	Large populations of <i>A. piceae</i> present on two mature <i>A. lasiocarpa</i> , no evidence of gouting, irregular branching at nodes. Two lower branches sampled, terminal buds with adelgids in buds, all life stages present	KJ445725
Rossland, 2 nd Av. and St. Paul St.	49.0794, -117.7971	2013	1060	Single <i>Abies lasiocarpa</i> on boulevard. Minor enlargement evident at nodes. Two lower branches sampled; all life stages present	KJ445726
Red Mountain Ski area, Olaus Way	49.1049, -117.8232	2013	1180	<i>Abies lasiocarpa</i> young regeneration, 2 trees sampled; branches with slightly enlarged nodes; lightly infested with adelgids	KJ445728
Red Mountain Ski area, Olaus Way	49.1049, -117.8232	2014	1180	Mature <i>Abies lasiocarpa</i> , one tree sampled; branches without enlarged nodes; lightly infested with adelgids	NS ¹
Rossland, Whiskey Trail	49.055, -117.794; 49.045, -117.791	2014	946 1128	<i>Abies lasiocarpa</i> , no gouting evident	KR260474 KR260470
Rossland, Larch Ridge Trail	49.158, -117.84	2014	1476	<i>Abies lasiocarpa</i> , no gouting evident	KR260469
Rossland, gravel pit	49.114, -117.823	2014	1216	<i>Abies lasiocarpa</i> , no gouting evident	KR260473
Rossland, Hwy 3B	49.094, -117.804	2014	1140	<i>Abies lasiocarpa</i> , gouting evident	KR260466 KR260472 KR260471 KR260472

NS¹ - not sampled for mtDNA as tree was from the same location as 2013 sequence KJ445728

The detection of *A. piceae* at Falls Creek in the Cascades Forest District (Table 2) was the first detection of the adelgid east of the Coast Mountains in B.C. The collection site is to the northeast of the previously most-eastern collections of *A. piceae* (Fig. 1, 3f), and appears to represent an eastward range expansion in B.C. Gouting was evident on *A. amabilis* infested by *A. piceae* at the sites sampled.

The implementation of provincial regulations defining regulated areas for *A. piceae* in B.C., which are supported by federal restrictions, has been successful in slowing the expansion of the infestation boundary of *A. piceae* in southwestern B.C. (Coulson and Witter 1984). As the range of the adelgid infesting Pacific silver fir and grand fir in the coastal forests of B.C. has slowly expanded (Fig. 3), the quarantine boundaries have been expanded and strategies have been developed to produce and distribute clean *Abies* seedlings for reforestation to prevent anthropogenic dispersal of *A. piceae*. Additionally, the growth, sale and distribution of ornamental *Abies* spp. within the province has been restricted, as have imports of all *Abies* spp. into B.C. to prevent redistribution of the pest to uninfested regions.

The records presented in this study document the known distribution of *A. piceae* in B.C. from historical survey records and recent detections. The records noted in Anon. (1938) indicate that *A. piceae* was introduced into southwestern B.C. on ornamental *Abies* before 1937, at least 20 years prior to the first reports of damage in North Vancouver (Silver 1959). Vyse (1971) states that the adelgid was first introduced into southwestern B.C. in about 1938, although no evidence is provided to document that assertion. The rapid expansion of the infested area detected in the first seven years after its initial discovery on the mainland (Fig. 3a, b) may not represent active dispersal and rapid range extensions of the pest from the infestations detected in 1957 (Silver 1959), but rather may have arisen from the detection of previously established populations introduced in the late 1930s. It is apparent from the historical records that the distribution of this destructive forest pest was actively expanding when federal survey efforts ceased after 1995 (Fig. 3f). The recent detections at Falls Creek demonstrate that this adelgid has continued to expand its range inland beyond the coastal forests during the last two decades.

The historical detections of the adelgid in mature stands have generally occurred well after its initial establishment in the stand—most often when visible damage such as gouting or mortality is discovered at any one location. Subsequent delimitation surveys beyond the initial find have often demonstrated that the pest is more widely distributed. The detection of *A. piceae* beyond the regulated area on Vancouver Island in 1993 led to the discovery of multiple infested stands well north of the boundary in subsequent years (Table 1). Negative survey records from northern Vancouver Island made after the discovery of populations on WTI (Fig. 3e, f) suggest that the adelgid was not yet active in those areas. However, difficulty of access has limited survey efforts on the adjacent mainland between WTI and known infested areas near Powell River first discovered in 1986. It is highly probable that *A. piceae* was established at least as far north as the mainland adjacent to WTI by 1987. Vyse (1971) estimated that over the two decades ending in 1987, the maximum range expansion of *A. piceae* to the northwest along mainland would reach the Campbell River area. The WTI infestations exceed the estimated maximum range expansion along the coast under the most pessimistic scenario discussed by Vyse (1971) by more than 50 km.

The first detections of *A. piceae* in Interior *A. lasiocarpa* stands near Rossland pose serious challenges to the quarantine management of this pest in B.C. The cryptic nature of *A. piceae* in the initial stages of an infestation makes detection of attacked stands and thus delimitation of boundaries for regulated areas extremely difficult. Although Mitchell (1966) noted that “significant gouting always accompanies the decline of subalpine fir, but is often not conspicuous because the trees die so quickly”, gouting was not apparent at many of the locations sampled near Rossland. The absence of such readily apparent symptoms of attack makes detection of infested trees considerably more difficult,

requiring the collection and microscopic examination of branch samples for presence of the adelgid under bud scales, at the base of staminate flowers, or at branch nodes. The massive numbers of white woolly masses associated with stem attack by *A. piceae* are more visually apparent than life stages associated with branch attack; however, stem attack usually begins high in the crown on subalpine fir and progresses down the stem (Mitchell 1966), also making the initial stages of such attacks difficult to detect. The presence of a native adelgid, *Pineus abietinus* (Underwood & Balch), attacking true firs in B.C. (Underwood and Balch 1964; Maw *et al.* 2000) further complicates recognition of stem attack by *A. piceae*, as the former species also develops dense populations on *Abies* stems.

Current quarantine regulations were developed to address potential sources of anthropogenic dispersal of *A. piceae* during harvest and reforestation activities in coastal forests, as well as to address that risk also posed by commercial distribution of potentially infested ornamental *Abies* spp. in urban areas. Regulations related to the movement of logs from within a regulated area will likely need to be modified, should quarantine restrictions be considered for management of this pest in Interior stands. Current regulations requiring the transport and storage of *Abies* logs in water are not feasible in the Interior of the province. Furthermore, current restrictions related to the production of seedlings for reforestation rely on the production of clean nursery stock in nurseries situated well beyond areas of known infestation to prevent infestation of the seedlings by aerially dispersed crawlers. To ensure that any sites used for the production of reforestation seedlings are free of populations of *A. piceae*, true firs present in the surrounding forests or cultivated as ornamentals near nurseries should be surveyed for the presence of *A. piceae*. Ideally, nursery stock should be produced in nurseries situated well beyond the range of native fir stands or ornamental plantings. Should this not be feasible, we recommend the use of sentinel plantings of *Abies* species that easily express persistent and apparent symptoms of infestation (e.g., gouting) by low numbers of *A. piceae*. Trees to be planted as sentinels should be grown from seed at nurseries remote from any populations of *A. piceae* in B.C.

Subalpine fir in southeastern B.C. is restricted to the upper elevations in the mountains, resulting in an extremely patchy distribution with very limited road access in most areas. Given the constraints noted above, development of a survey strategy will be extremely difficult. Should surveys for *A. piceae* be undertaken in subalpine fir forests, we recommend that presence of the pest be documented with properly preserved samples of life stages suitable for both morphological (i.e., preserved in 70% ethanol) and molecular identification (preserved in 95% ethanol). These samples are also essential to separate attack by *Adelges piceae* from that of the non-damaging native species, *Pineus abietinus* (Cook *et al.* 2010).

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Table 4
High-elevation locations at which *Abies lasiocarpa* was sampled in southern British Columbia and no evidence of *Adelges piceae* infestations was detected in 2014.

Location	Latitude (Dec. Deg.)	Longitude (Dec. Deg.)	Elevation (m)	No of trees	Comments
Mt. Baldy	49.1534	-119.2393	1780	5	2-4 m regeneration; no visible signs of gouting
Mt. Kobau	49.1147	-119.6749	1860	10	Scattered mortality in mature trees at summit; yellowing foliage; no visible signs of gouting or stem attack. Heavy populations of Pseudococcidae on lower branches
Apex Mountain Ski Hill	49.3925	-119.9037	1690	10	Mature trees; no visible signs of gouting
Big White Ski Hill (base)	49.7259	-118.9227	1820	10	2-4 m regeneration; no visible signs of gouting
Glenmerry FSR	49.2348	-117.9646	1340	10	Advanced regeneration; no evidence of gouting
Nancy Green Lk Prov Pk	49.2596	-117.9414	1274	1	Roadside tree; no visible signs of gouting
Bridal Lake	49.0621	-117.0387	1790	10	Advanced regeneration; no evidence of gouting
Maryland FSR	49.0650	-116.9155	1011	10	Advanced regeneration to 4m in height; no evidence of gouting; immature Coccidae under bud scales at nodes of a single tree.
Dodge Creek FSR	49.0113	-116.6370	1425	10	Mature trees with no evidence of gouting
Kimberly, North Star Ski Hill	49.6833	-116.0090	1325	5	Advanced regeneration at base of ski hill; no evidence of gouting

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Pollen preferences of two species of *Andrena* in British Columbia's oak-savannah ecosystem

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ABSTRACT

Although understanding the requirements of species is an essential component of their conservation, the extent of dietary specialisation is unknown for most pollinators in Canada. In this paper, we investigate pollen preference of two bees, *Andrena angustitarsata* Vierick and *A. auricoma* Smith [Hymenoptera: Andrenidae]. Both species range widely throughout Western North America and associated floral records are diverse. However, these species were primarily associated with spring-blooming Apiaceae in the oak-savannah ecosystem of Vancouver Island, BC, specifically *Lomatium utriculatum* [Nutt. ex Torr. & A. Gray] J.M. Coult. & Rose, *L. nudicaule* [Pursh] J.M. Coult. & Rose, and *Sanicula crassicaulis* Poepp ex. DC. Floral visit records and scopal pollen composition for these species from two regions on Vancouver Island indicate dietary specialisation in oak-savannah habitats where Apiaceae are present. Both species were also caught in low abundances in residential gardens where Apiaceae were scarce, sometimes on unrelated plants with inflorescence morphology similar (to our eyes) to Apiaceae. Further study of these species is needed to understand whether preferences observed locally in BC exist elsewhere in their range. Our findings contribute to understanding pollen preference in natural and urban areas, and highlight an important factor to consider for species-specific conservation action in a highly sensitive fragmented ecosystem.

Key Words: Andrenidae, Apoidea, oligolecty, pollen preference

INTRODUCTION

Relationships between flowering plants and bees (Hymenoptera: Apoidea, Apiformes) range from extreme pollen specialisation, or oligolecty, to extreme generalisation, or polylecty. Perhaps as many as half of all non-parasitic bee species exhibit dietary specialisation at some level, but for most bees the extent of specialisation is unknown (Cane and Sipes 2006; Michener 2007). Bee species are considered to exhibit narrow oligolecty if females consistently provision offspring with pollen from a small related clade of plants (typically genus level; Linsley and MacSwain 1958; Müller 1996; Cane and Sipes 2006). Broad oligolecty could potentially comprise a larger number of related plant species or genera, but differs from polylecty in that many to most of the available pollen sources are not utilized (Cane and Sipes 2006). Facultative oligolecty occurs in some bee species, which normally specialize on a host plant, but may use non-host pollen when the usual host is unavailable (e.g., Williams 2003; Sipes and Tepedino 2005). Use of non-host pollen by oligolectic species can sometimes have substantial fitness consequences (Praz et al. 2008a), which may have conservation implications and explain why specialists tend to be more sensitive to disturbance (Elle et al. 2012).

Evaluating specialisation can be challenging. The identity of plant species from which bees are collected has frequently been used to describe foraging preferences, but we have known since the landmark work of Charles Robertson that bees visit a broader array of flowers for nectar than for pollen (Robertson 1925). Similarly, foraging preferences of male bees, which do not provision nests with pollen (they forage only for

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nectar), should not be considered when evaluating dietary specialisation. Floral records that do not include information on gender and behaviour simply have limited utility (Cane and Sipes 2006). Instead, it is important to consider not only constancy of floral visits by female non-parasitic bees, but also the composition of pollen they collect for offspring provisioning.

Habitat loss and fragmentation is predicted to have negative effects on biodiversity unless organisms can use the surrounding landscape (or “matrix”) for food or nesting resources (Dunning *et al.* 1992; Fahrig 2001; Wray and Elle 2015). Dietary specialists are expected to be negatively impacted by habitat conversion because the plant species upon which they rely are generally not available in the non-habitat matrix. In studies examining the effects of habitat loss on pollinating insects, specialists consistently decline with reduction in natural habitat (Steffan-Dewenter and Tscharntke 2000; Burkle *et al.* 2013). Britain and the Netherlands experienced parallel declines in bee-dependent plants and specialist bees (Biesmeijer *et al.* 2006), in some cases, because the plant species required by the bees have been locally extirpated. Documenting dietary specialisation, therefore, is important for recognition of threats to bees of particular conservation concern, the plants upon which they rely for offspring provisioning, and potentially how reproductive output of those plants would be impacted by pollinator loss. Such research can be especially interesting in human-dominated landscapes where host plants may vary in availability (MacIvor *et al.* 2014).

We investigated potential pollen specialisation of two mining bees, *Andrena angustitarsata* Vierick and *A. auricoma* Smith (Hymenoptera: Andrenidae) in a highly fragmented oak-savannah ecosystem. Less than 5% of this habitat remains due to residential expansion, agricultural development, and the introduction of invasive species (Fuchs 2001). We noticed that collections of these bees at our study sites were primarily from two species of *Lomatium* (Apiaceae), spring gold (*L. utriculatum* [Nutt. ex Torr. & A. Gray] J.M. Coult. & Rose) and Indian consumption plant (*L. nudicaule* [Pursh] J.M. Coult. & Rose), suggesting the bees may be oligolectic. However, the range of both bees comprises the majority of Western North America, and the floral records included in species descriptions are quite diverse (LaBerge and Ribble 1975; LaBerge 1989). We have also more recently been working in urban and suburban neighborhoods near oak-savannah fragments, where the host plants are not available (Wray and Elle 2015). We were therefore interested in documenting the proportion of visits by *A. angustitarsata* and *A. auricoma* females to Apiaceae (at our field sites, the two *Lomatium* species and *Sanicula crassicaulis* Poepp ex. DC) vs. other plant species, and the composition of pollen collected by females for nest provisioning, to better understand whether these *Andrena* spp. are facultative oligoleges in our region.

METHODS

As part of two larger research projects, bees were collected directly from flowers in oak-savannah fragments of the Cowichan Valley (“North OS”, 2008–2010), and oak-savannah fragments and urban residential gardens on the Saanich Peninsula (“South OS”, “South Gardens”, 2012) of Vancouver Island, British Columbia (Fig. 1, Gielens 2012; Wray and Elle 2015). Bees collected from flowers of a single plant species (or genus in gardens, where botanical cultivars were common) were euthanized in the same cyanide tube during the duration of a sample period. For North OS sites, two 15-minute plant-species-specific samples were collected on each day sampling occurred (Gielens 2012). In South OS sites and gardens, two 30-minute samples were taken on each day of sampling, with collections made from any plant species in flower, although collected insects were still kept separate by plant species (Wray and Elle 2015). For both studies, collection dates comprised the majority of the flowering season of our sites, from April to July. Bees of the species of interest were collected from 11 April to 27 June, depending on year and site, with the majority collected in May. Because of the variability in

numbers of bees collected at different sites, we calculated the proportion of *A. angustitarsata* and *A. auricoma* collected on different plant species within a geographic region (i.e., North OS, South OS, South Gardens), rather than by sites within a region.

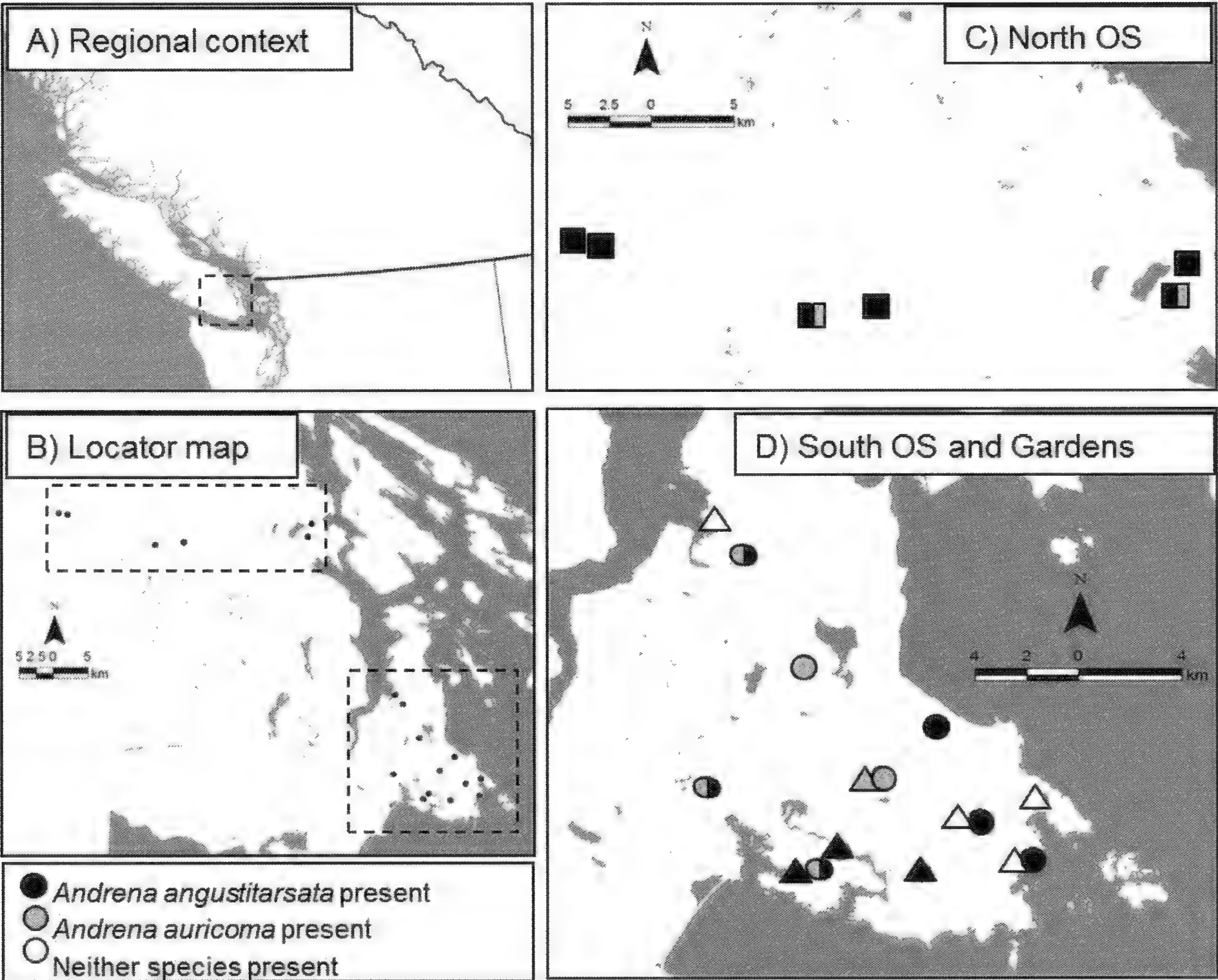


Figure 1. Maps illustrating locations of study sites and which focal species were collected at each. Land is white and ocean is grey. Dashed boxes indicate the area magnified in subsequent panels. A) Vancouver Island in the broader regional context of British Columbia (from iMapBC, <http://maps.gov.bc.ca/ess/sv/imapbc/>). B) Locator map of southern Vancouver Island, with two regions of study outlined. C) North Oak-Savannah (OS) sites (squares). C) South OS (circles) and South Gardens (triangles). Symbol colour indicates species collected (black: *Andrena angustitarsta*; grey: *A. auricoma*; split symbols: both species; white: neither).

Female bees were visually examined for the presence of scopal pollen, and a random sample of those individuals was included in the analysis. Only pollen from the tibial scopa and propodeal corbicula (i.e., pollen-carrying regions of *Andrena*) was analysed because our intention was to investigate the pollen collected for offspring provisioning. Using pollen from these areas also reduced the likelihood of any contamination by non-scopal pollen from our cyanide tubes. The pollen load from the scopa of one leg was scraped into a 1-mL centrifuge tube with a sterilized needle. In addition, the leg and propodeum were washed with 1mL of 70% alcohol. The pollen/alcohol mixture was centrifuged at 3500 rpm for 5 min, discarding the supernatant after centrifuging. Pollen pellets were left in centrifuge tubes to dry for 30 min. We added 10uL Caberla solution (6mL glycerin, 12mL 95% ethyl alcohol, 18mL distilled water, 50mg basic fuchsin stain [pararosanine]), and 10uL glycerin to the pollen pellet, and tubes were agitated to suspend pollen in solution. We transferred the entire 20-uL sample to a glass slide, covered the pollen solution with a cover slip, and used clear nail polish to seal the edges.

We identified pollen under a light microscope at 400X magnification with the assistance of reference pollen slides made from flowers collected from our sites and from the SFU Palynology Lab. Pollen grains were identified to “type” at the lowest possible taxonomic level—species, genus, or family (Table 1). Pollen morphology is often conserved within a genus or family and so identification to species is frequently not possible. When pollen was identified to genus or family, but could not be unambiguously assigned to species using our reference collection, we inferred likely species based on vegetation-sampling data from our sites (Gielens 2012; Wray and Elle 2015) and include a note in the table. Pollen grains were counted in four random “transects” of the cover slip (as in Müller 1996). Samples with insufficient pollen (<50 grains) were not included in results. Any pollen types that contributed less than five percent to the total pollen count on a slide were deleted to account for the possibility of trace contamination (Cane and Sipes 2006). However when averaged across all samples within a geographic region (North OS, South OS, South Gardens), some pollen types still contributed less than five percent to the total pollen count across all slides from that region. In addition to presenting the average proportion of pollen types for different regions, we include information on the incidence of “pure” loads (100% host pollen once trace contaminants comprising <5% removed, as in Cane and Sipes 2006)

Table 1

Description of pollen types, including the family, genera, and species combined into the different types. When pollen could not be attributed to a particular plant species using our reference collection, likely species identities listed in the table are based on our vegetation surveys, as indicated.

Pollen type	Family/genera/species included
Apiaceae-1	<i>Lomatium utriculatum</i> , <i>L. nudicaule</i>
Apiaceae-2	<i>Sanicula crassicaulis</i>
Asteraceae	Many species possible. Based on our vegetation surveys, this pollen type could include <i>Achillea millefolium</i> , <i>Balsamorhiza deltoidea</i> , <i>Bellis perennis</i> , <i>Eriophyllum lanatum</i> , <i>Hypochaeris radicata</i> , and/or <i>Taraxacum officinale</i>
Brassicaceae	Likely <i>Brassica</i> spp., based on our vegetation survey
Rhamnaceae	<i>Ceanothus</i> spp. (probably <i>C. thyrsiflorus</i> cv. “Victoria” as this was commonly planted in gardens)
Rosaceae-1	<i>Sorbus</i> spp. (usually <i>S. aucuparia</i> , according to our vegetation survey)
Rosaceae-2	<i>Rosa nutkana</i>
<i>Trifolium pratense</i>	<i>Trifolium pratense</i>
<i>Trifolium repens</i>	<i>Trifolium repens</i>
Other	Caryophyllaceae, Ranunculaceae, Unknown-1

RESULTS

Two pollen types that could be assigned via morphology to Apiaceae were detected in our samples. Based on our reference collection, Apiaceae-1 is consistent with *Lomatium* spp., and so could be either *L. utriculatum* or *L. nudicale*. The larger grains of Apiaceae-2

are consistent with *Sanicula crassicaulis* (Fig. 2). Inflorescence morphology for these three species is also shown in Figure 2, and all are comprised of dense heads of small flowers.

***Andrena angustitarsata*.** We collected a total of 141 females of this species from oak-savannah fragments in the Cowichan Valley (“North OS”) and on the Saanich peninsula (“South OS”), and from residential gardens on the Saanich Peninsula (“South Gardens”; Table 2). Most were collected from flowers of Apiaceae; 89% in North OS sites, and 98% in the South OS sites (Table 2). In South Gardens, only 11 *A. angustitarsata* were collected, none from flowers of Apiaceae. No plants from this family were blooming in residential gardens during the spring flight period of this bee, although some Apiaceae genera bloom in gardens in July and August (*Astrantia*, *Eryngium*, *Foeniculum*, *Pastinaca*). In gardens, bees were collected from a number of plant species with diverse floral morphologies, including small flowers densely arranged in compact heads or umbels (e.g., *Ceanothus* Linnaeus [Rhamnaceae], *Sorbus* Linnaeus [Rosaceae], *Spiraea* Linnaeus [Rosaceae]) and simple flowers with easily accessible pollen and nectar rewards (*Brassica* spp. Linnaeus [Brassicaceae]).

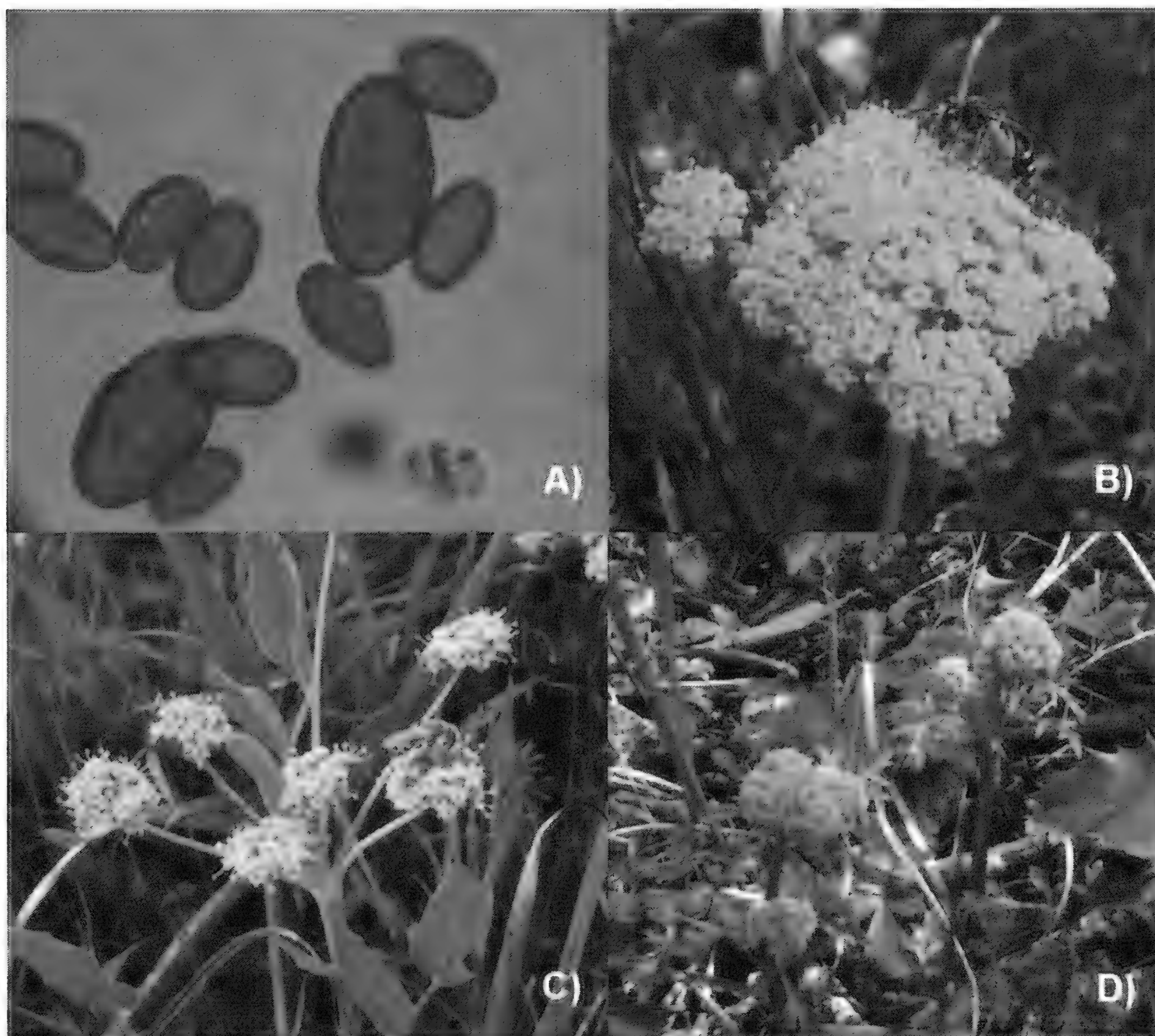


Figure 2. Pollen grains and inflorescence architecture of focal plant species: A) Smaller pollen grains are Apiaceae-1 (includes both *Lomatium utriculatum* and *L. nudicaule*), and larger grains are Apiaceae-2 (*Sanicula crassicaulis*); B) *L. utriculatum*; C) *L. nudicaule*; D) *S. crassicaulis*.

We investigated composition of actively collected pollen (from scopae and corbiculae) for 73 bees (Table 2). Pollen grains counted per slide ranged from 117 to 11,225 grains, with an average of 2,334 grains per slide. Across all North OS sites, *A. angustitarsata* pollen loads were comprised primarily of Apiaceae-1 (83%, *Lomatium* spp.), followed by Rosaceae-1 (13%, *Sorbus* spp.). Brassicaceae and Rosaceae-2 each contributed less than 2% to the total pollen sample, while unknown pollen types combined into the “Other” category were less than 1% (Fig. 3A). Of the 34 North OS bees included in the pollen analysis, 16 (47%) had “pure” pollen loads of Apiaceae-1.

In South OS sites, pollen was almost exclusively Apiaceae-1 (98%). Brassicaceae, Rosaceae-1 and Other each contributed less than 1% to the total pollen composition (Fig. 3C). Of the 31 South OS bees included in the pollen analysis, 28 (90%) had “pure” pollen loads.

In South Garden sites, there was no Apiaceae pollen in samples that could not be attributed to contamination (e.g., 1-2 grains out of several hundred counted per sample). Instead, samples were comprised largely of Rosaceae-1 (42%), Brassicaceae (21%), Rhamnaceae (17%), and *Trifolium repens* (13%, Fig. 3E). *Trifolium pratense* contributed less than four percent to the total, and Other types less than one percent.

***Andrena auricoma*.** There were a total of 49 female *A. auricoma* collected from oak-savannah fragments (19 North OS sites, 30 South OS sites). Only two females were collected from South Garden sites. Females were predominantly collected from flowers of Apiaceae (95% in North OS, 83% in South OS), the majority from plants in the genus *Lomatium* (Table 2).

Pollen composition was investigated for a total of 32 female bees. We counted between 58 and 4,984 grains per slide, with an average of 1,465 grains. The two females collected from South Garden sites were not included in pollen analysis, due to low numbers of pollen grains (<50 grains counted). In both OS regions (North and South), Apiaceae-1 was the dominant pollen type (North: 61%, South: 75%) followed by Apiaceae-2 (North: 37%, South: 19%; Fig. 3B, D). In North OS sites, Rosaceae-1 and *Trifolium repens* each contributed less than 2% (Fig. 3B). In South OS sites, Rosaceae-1 contributed 5% to total pollen counts, and Asteraceae less than 1% (Fig. 3D).

Of the 11 female bees included from North OS, 9 of 11 (82%) had “pure” pollen loads (combined Apiaceae 1 and 2). Of the 19 females included from South OS sites, 17 (89%) had “pure” loads.

DISCUSSION

We found evidence of preference for Apiaceae pollen for two bee species in oak-savannah habitats on Vancouver Island. Female *Andrena angustitarsata* and *A. auricoma* predominantly visit *Lomatium* spp. and *Sanicula crassicaulis* in this region, and pollen collected to provision nests is largely from the Apiaceae. Dietary specialization is normally considered a characteristic of species, rather than something that varies across a species’ range. The published floral host records for these species are diverse (LaBerge and Ribble 1975; LaBerge 1989), suggesting polylecty. Further evaluation of pollen collection behaviour throughout the ranges of these species would be useful, as it would allow visits for nectar or by male bees to be distinguished from visits by females actively provisioning nests with pollen. Confirmation of observed preferences, an evaluation of preferences in other parts of the species’ ranges, and clarification of the ecological conditions that promote these preferences in otherwise polylectic bees is clearly required.

It is unlikely that these species are simply foraging on Apiaceae due to disproportionate availability of Apiaceae flowers. For example, during the spring bloom of *Lomatium* spp. and *Sanicula crassicaulis*, these plants combined contributed less than 5% to the total cover of blooming flowers recorded in surveys of South OS sites (Wray and Elle 2015). In addition, other pollen sources (e.g., common camas, *Camassia quamash* [Pursh] Greene [Asparagaceae]; great camas, *Camassia leichtlinii* [Baker] S.

Watson [Asparagaceae]) were abundant and available and are consistently used by other solitary mining bees, mason bees, social bumble bees, and honey bees (Gielens 2012; Wray and Elle 2015). Instead, our results indicate a preference for Apiaceae for these two mining bees, particularly plants in the genus *Lomatium*.

Table 2
Total numbers of *Andrena angustitarsata* and *Andrena auricoma* collected from different study locations. Of the total collected, we state the number (and percentage, in brackets) of all collected bees that were caught foraging on plants in the Apiaceae, and within Apiaceae, the total that were collected from *Lomatium utriculatum* and *L. nudicaule* combined. The number randomly chosen for pollen analysis is also indicated.

	<i>Andrena angustitarsata</i>			<i>Andrena auricoma</i>	
	North OS	South OS	South Gardens	North OS	South OS
Total number collected	99	42	11	19	30
Collected from Apiaceae	88 (88.9%)	41 (97.6%)	0 (0%)	18 (94.7%)	25 (83.3%)
Collected from <i>Lomatium</i>	85 (85.9%)	40 (95.2%)	0 (0%)	11 (57.9%)	21 (70.0%)
Included in pollen analysis	34	31	8	11	21

Documenting dietary specialisation and/or preference is highly dependent on considering pollinator sex, and evaluating pollen loads used for rearing offspring (Cane and Sipes 2006). *Andrena angustitarsata* is considered a polylectic species, with over 2000 females and 1400 males collected on plants from 61 genera in 24 families in Western North America and included in the revision by LaBerge (1989). These collections include records from multiple species of *Lomatium* and *Sanicula*, as well as *Ceanothus*, *Ranunculus* Linnaeus (Ranunculaceae), *Salix* Linnaeus (Salicaceae), *Brassica*, and *Spiraea*. Similarly, *Andrena auricoma* has been collected from flowers of 44 genera and is also documented to be polylectic (LaBerge and Ribble 1975). The most frequent collections in decreasing order were from *Ranunculus*, *Descurainaea* Webb and Berth (Brassicaceae), *Salix*, and *Potentilla* Linnaeus (Rosaceae). However, these collection records do not indicate the sex of the specimen, foraging behaviour (nectar vs. pollen foraging), nor has the pollen collected by female bees been examined. Without this information, one cannot confidently assume these species are polylectic or oligolectic. As such, it is difficult to determine whether Apiaceae preference by *A. angustitarsata* and *A. auricoma* is simply localised to our study region of oak-savannah habitat on Vancouver Island, or if it may be more widespread and present in other habitat types within the broad range of these species. In our region, at least, our data suggest the species should be considered facultative oligoleges, *sensu* Cane and Sipes (2006).

Specialist bees are predicted to be more sensitive to the effects of habitat loss and fragmentation (Davies *et al.* 2000; Henle *et al.* 2004), and our study species were caught in low abundances outside of natural oak-savannah habitat (*A. angustitarsata*: 11 total at three South Garden sites, average 1.4/site out of eight total South Garden sites sampled; *A. auricoma*: 2 total at a single South Garden site, average 0.25/site). The fitness consequences for these bees in gardens are not known, but for other species, the

consequences vary. For example, Praz *et al.* (2008a) found in some oligolectic species, larvae failed to develop on non-host pollen; Haider *et al.* (2013) found within- and among-population variation in offspring development on non-host pollen; and Williams (2003) found no effect on development for specialist larvae reared on non-host pollen. However, successful development on non-host pollen does not necessarily change foraging preference of adults, as Praz *et al.* (2008b) found that larvae successfully reared on non-host pollen preferred their normal host in choice-tests as adults. We do not know if non-Apiaceae pollen would support successful offspring production by these two species in our region, or if such offspring would subsequently maintain oligolecty; this should be studied.

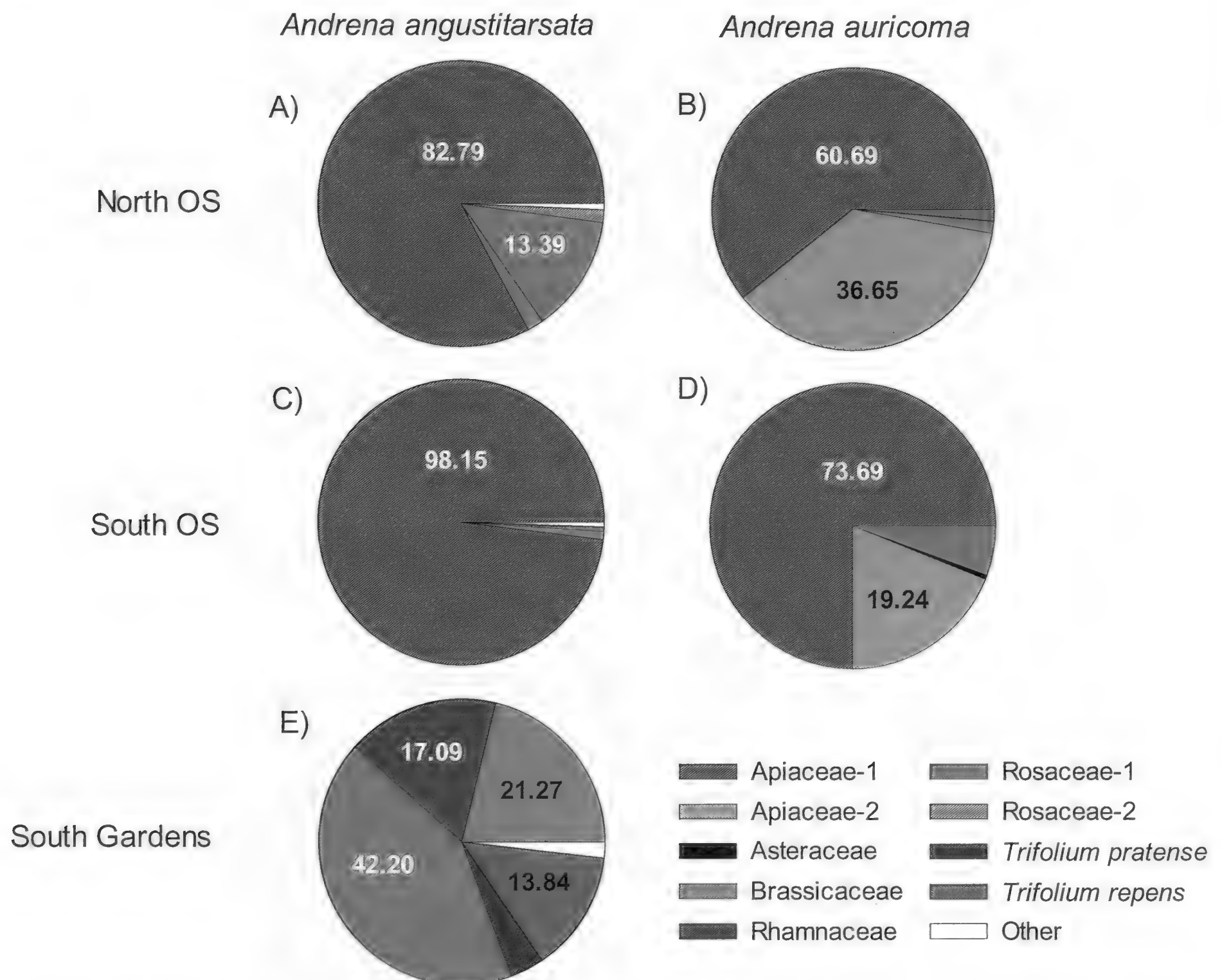


Figure 3. Average proportional pollen composition for *Andrena angustitarsata* and *A. auricoma* in North OS (A, B), South OS (C, D), and South Garden sites (E). Numbers in pie sections indicate the percentage a pollen type contributed to the total pollen count.

We found it curious that bees in gardens were collecting pollen from non-host plants that appear (to our eyes) morphologically similar to Apiaceae. *Lomatium utriculatum* has small flowers arranged in flat umbels, and *L. nudicaule* and *S. crassicaulis* have small flowers arranged in spherical clusters (Fig. 2). In gardens, *Sorbus* and *Ceanothus* comprised a large proportion of the diet of the few *A. angustitarsata* collected, and have similar inflorescence architecture to the native host plants. Pollen chemistry has been invoked as an important cue for oligolectic species (e.g., Müller and Kuhlman 2008), but floral morphology may also be important if specialists are limited in their ability to extract resources from flowers with morphology different than that of hosts (Thorp 1979; Müller 1996; Williams 2003). The apparent similarity of visual cues between host and

non-host flowers suggests an investigation of foraging decisions when Apiaceae are unavailable would be worth pursuing in future studies.

Our study provides only a baseline of information on pollen preference in *A. angustitarsata* and *A. auricoma* within BC, and clearly further assessment of pollen provisioning behaviour needs to be done throughout these species' ranges. Our data do suggest that, in the geographically restricted oak-savannah ecosystem on Vancouver Island, *L. utriculatum*, *L. nudicaule*, and *Sanicula crassicaulis* provide vital resources for *A. angustitarsata* and *A. auricoma*. Urban residential gardens in our area support a diversity of bees, including two species in the Megachilidae that are Asteraceae specialists (*Megachile perihirta* Cockerell (Megachilidae) and *Osmia coloradensis* Cresson (Megachilidae); Wray and Elle 2015). Homeowners could consider planting native oak-savannah wildflowers to sustain these, and other specialist bees.

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Relative efficacies of sticky yellow rectangles against three *Rhagoletis* fly species (Diptera: Tephritidae) in Washington State and possible role of adhesives

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ABSTRACT

Sticky yellow rectangle traps are used to monitor various pest *Rhagoletis* flies (Diptera: Tephritidae), but it is unclear if relative efficacies of these traps differ with fly species. Here, the main objective was to identify the most efficacious of five commercial sticky yellow rectangles baited with ammonium carbonate against western cherry fruit fly, *R. indifferens* Curran, apple maggot fly, *R. pomonella* (Walsh), and walnut husk fly, *R. completa* Cresson, in Washington State, U.S.A. Two plastic yellow sticky strips (PL1 and PL2) supplemented with Tanglefoot adhesive and three sticky yellow cardboards, the Pherocon AM (PA1), Multigard AM (PA2) and Alpha Scents Yellow Card (PA3), were tested. Across all three species, the PL1 and PL2 + Tanglefoot generally caught the most flies, the PA3 sometimes caught more than the PA1, and all caught more than the PA2. Adding Tanglefoot to the PA1 did not make the trap as efficacious as the PL1 + Tanglefoot against *R. indifferens*, but it did against *R. pomonella* and *R. completa*. Results suggest the plastic rectangles tested here are better than standard cardboard rectangles for capturing high numbers of all three *Rhagoletis* species, implying they should be the rectangles of choice for monitoring these flies. Results also suggest that similar trap efficacies against the three species may have different underlying causes.

Key Words: *Rhagoletis indifferens*, *Rhagoletis pomonella*, *Rhagoletis completa*, yellow plastic traps, yellow cardboard traps, Tanglefoot® adhesive

INTRODUCTION

Traps are used to monitor and detect various pest *Rhagoletis* flies (Diptera: Tephritidae) as a first step in a multi-pronged approach for protecting fruit commodities. Of all the different trap types developed, sticky yellow rectangles baited with ammonia compounds, in particular ammonium carbonate, remain the most widely used against these flies in North America (e.g., Riedl *et al.* 1989; Liburd *et al.* 2001; Yee *et al.* 2012). These traps are commercially available, flat, light, easy to store and deploy, and the dark flies are easy to see on and remove from them. Other commercial sticky traps used in North America or Europe are red or green spheres, the Ladd trap (AliNiazee *et al.* 1987; Riedl *et al.* 1989; Jones and Davis 1989), and the Rebell trap (Remund and Boller 1978). Non-commercial, experimental sticky traps include yellow spheres (AliNiazee 1981), a bell trap (Burditt 1988), and cylinder traps (Opp *et al.* 2003).

Efficacies (that is, how well a trap performs in controlled experiments relative to other traps) between rectangle and some other trap types differ among *Rhagoletis* species (e.g., Prokopy and Hauschild 1979; Liburd *et al.*, 2001; Lampe *et al.* 2005). For example, against European cherry fruit fly, *R. cerasi* (L.), three-dimensional yellow Rebell traps are more efficacious than Pherocon AM traps, whereas the reverse is true for eastern cherry fruit fly, *R. cingulata* (Loew) (Katsoyannos *et al.* 2000; Lampe *et al.* 2005).

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However, which yellow rectangles are most efficacious and whether efficacies of those rectangles differ among *Rhagoletis* species have not been well studied.

In particular, sticky yellow plastic rectangles that catch more western cherry fruit fly, *R. indifferens* Curran, than conventional sticky yellow cardboard rectangles in Washington State, U.S.A. (Yee 2014), have not been tested against other *Rhagoletis* species. It can be predicted that plastic yellow rectangles are efficacious against them as well, based on similarities in spectral sensitivities in representative species and fly responses to color (Prokopy 1968; Agee *et al.* 1982; Agee 1985). The availability of different commercial yellow rectangles presents an opportunity to test this hypothesis. Rejection of this hypothesis could lead to work identifying factors responsible for trap efficacy and thus more species-specific traps.

In western North America where fruit commodities are of high economic value, trapping is critical for quarantine and control measures against three *Rhagoletis* species. In Washington State and other northwestern U.S. states, as well as in British Columbia, Canada, *R. indifferens* and apple maggot fly, *R. pomonella* (Walsh), are major quarantine pests of cherries (*Prunus* spp.) and apple (*Malus domestica* Borkhausen), respectively. In California, walnut husk fly, *R. completa* Cresson, is a major pest of walnuts (*Juglans* spp.). Annually, in Washington State, cherries are valued at ~\$US300–\$400 million (National Agricultural Statistics Service 2013a) and apples at ~\$US1.5 billion (National Agricultural Statistics Service 2012); in California, walnuts are valued at ~\$US1.3 billion (National Agricultural Statistics Service 2013b).

In this study, the main objective was to identify the most efficacious of five commercial sticky yellow rectangles baited with ammonium carbonate against *R. indifferens*, *R. pomonella*, and *R. completa* in Washington State. The hypothesis that relative efficacies of these traps against all three species are similar was tested. A secondary objective was to determine which factors could affect their efficacies. In particular, traps were supplemented with Tanglefoot® adhesive (Contech Enterprises, Inc., Victoria, B.C., Canada) to determine if this can increase the efficacy of a trap, as adhesive type effects on fly captures may vary (Yee 2011).

MATERIALS AND METHODS

Five-trap comparisons. Five commercial sticky yellow rectangles were tested in the first set of experiments (Table 1, Fig. 1). The two plastic traps were the Agri-Sense Yellow Sticky Strip (PL1) and the Olson Yellow Sticky Strip (PL2) (Agri-Sense-BCS, South Wales, U.K., and Olson Products, Medina, OH, U.S.A., respectively), both covered with pressure-sensitive adhesives. These adhesives are thin, solvent-/water-free tacky materials unlike conventional thick, Vaseline-like adhesives. Both traps were 14 × 23 cm. The pressure-sensitive adhesives on the traps were supplemented with Tanglefoot® (Tangle-Trap™ Insect Trap Tropical Formula). Tanglefoot was added, because it is known from previous tests (Yee 2014; W. L.Y., unpublished) that the pressure sensitive adhesives on these traps can lose stickiness within 2–3 weeks. More importantly, the PL1, as received from the manufacturer, had variable amounts of pressure-sensitive adhesive, and occasional lots were not sticky. About 10 g of Tanglefoot (5 g each side) was spread onto each plastic trap.

The three cardboard or paper traps were the Pherocon AM (PA1), used for the last 40 years against *Rhagoletis* flies (e.g., Prokopy and Hauschild 1979; Riedl *et al.* 1989; Liburd *et al.* 2001), the Multigard AM (PA2; it or its variations have been available since at least 1994 [Katsoyannos *et al.* 2000]), and the Alpha Scents Yellow Card (PA3; available within the last six years [Yee 2011]; Table 1, Fig. 1). These three traps were initially tested without adding Tanglefoot to them, because they were assumed to retain their tackiness over test trap durations of ≤4 weeks. The sticky adhesives on the PA1 and PA2 were Vaseline-like, but differed from the Tanglefoot (compositions of commercial adhesives are proprietary). About 5.0 g and 5.5 g of sticky adhesives were present on the

Table 1
Five commercial sticky yellow rectangles tested against *Rhagoletis* flies in Washington State, U.S.A.

Sticky area			Mean color space values ± SE ^f			Light passage ^g
Trap ^a	Form	Size (cm)	(cm ²) ^e	Thickness	L* a* b*	
PL1 + Tanglefoot ^b	1 sheet	23 × 14	596	0.33	76.83 ± 0.22 -9.39 ± 0.07 63.22 ± 0.67	4,736
PL2 + Tanglefoot ^b	1 sheet	23 × 14	596	0.30	81.09 ± 0.62 -13.68 ± 0.11 57.55 ± 0.67	2,691
PA1 ^c	folded	23 × 14	407	1.22	88.77 ± 0.60 -6.41 ± 0.14 77.56 ± 0.89	54
PA2 ^c	folded	23.5 × 14	313	1.23	98.11 ± 0.18 -26.80 ± 0.29 81.22 ± 1.57	43
PA3 ^d	1 sheet	20 × 14	478	0.48	92.86 ± 0.57 -15.02 ± 0.13 68.39 ± 0.89	474

^aPL, plastic; PA, cardboard = paper. ^bTanglefoot (Contech Enterprises, Inc., Victoria, B.C., Canada) spread over pressure-sensitive adhesive on trap supplied by manufacturer; ~10 g per trap. ^cAdhesives of proprietary formulations different from Tanglefoot: 5 g and 5.5 g on PA1 and PA2, respectively. ^dHot-melt pressure-sensitive adhesive. ^eFrom manufacturer: total of two sides of traps; excludes borders without adhesive. ^fMeasured over sticky surface; five traps. ^gLm/m², measured with white light of 9,688 lm/m² on one side of trap; room light of 1,076 lm/m² (Yee 2014). Manufacturers: PL1: Agri-Sense-BCS, South Wales, U.K.; PL2: Olson Products, Medina, OH, U.S.A.; PA1: Trécé, Adair, OK, U.S.A.; PA2: Scentry Biologicals, Billings, MT, U.S.A.; PA3: Alpha Scents, West Linn, OR, U.S.A.

PA1 and PA2, respectively. The sticky material on PA3 was a hot-melt pressure-sensitive adhesive that was tackier than the pressure-sensitive adhesive on the PL1 (Yee 2011).

A vial containing 10 g of ammonium carbonate (Keystone Universal, Melvindale, MI, U.S.A.) with a plastic lid and two 1-mm holes was hung ~1 cm above each trap.

Study sites and experimental types in five-trap comparisons. All sites were located in central or western Washington State (WA). Sites were unmanaged orchards, homeowners' yards, or wild habitats. Twelve tests were conducted between May and September 2014 in sweet cherry (*Prunus avium* (L.) L.), apple, black hawthorn (*Crataegus douglasii* Lindley), and English walnut trees (*Juglans regia* (L.); Table 2). For each fly species, three to five tests were conducted, each using a randomized complete block design with three to five replicate blocks.

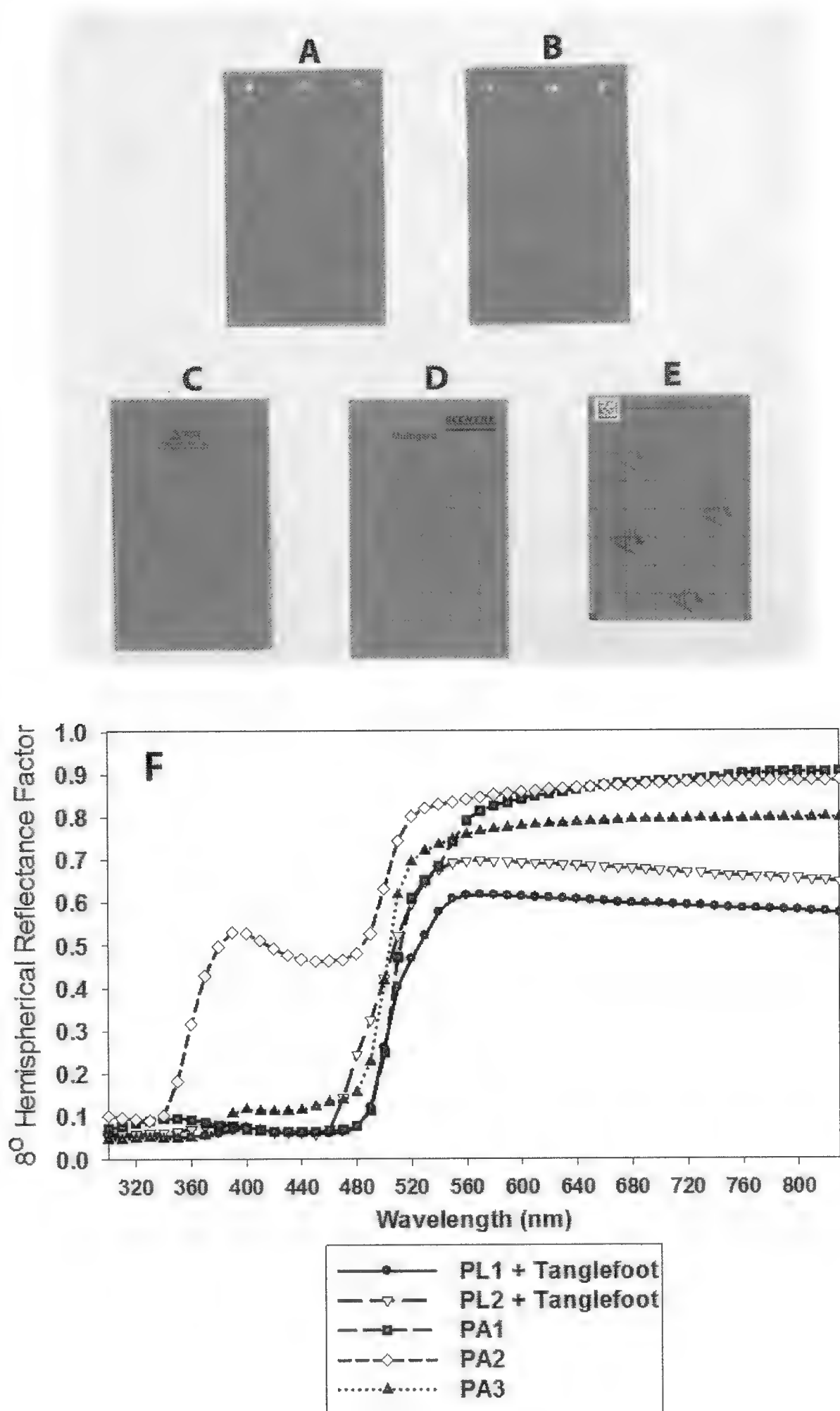


Figure 1. Yellow rectangle traps used in study: (A) PL1 + Tanglefoot, (B) PL2 + Tanglefoot, (C) PA1, (D) PA2; (E) PA3; all to same scale; (F) reflectance curves of the five traps (Perkin-Elmer Lambda 9UV-Vis-NIR Spectrometer Ser. No. 1611; Avian Technologies LLC, Sunapee, NH).

A block was a defined location comprising a set of trees, a single tree, or a sector of a tree containing all five trap types and within which all trap types were rotated to further reduce spatial effects. The number of blocks equaled the number of replicate traps. Availability of trees and layout of trees determined the number of replicates and possible blocking schemes, as no sets of trees in the field are neatly arranged like trees in orchards.

One trap per tree was set up in five-tree blocks when at least 25 trees (five trap types \times five replicate trees) were available to use at a site. Here, each trap was spaced 3–5 m apart, depending on inter-tree distances. This scheme was used for *R. indifferens* and *R. pomonella* (Table 2). When only about five large trees were present, all five trap types were placed in one tree; each tree was a block. All traps were placed in the south half of a tree, \sim 2 m apart. This scheme was used for *R. indifferens*, *R. pomonella*, and *R. completa* (Table 2). Two or three blocks per tree were set up when there were only two large, 15–18 m wide walnut trees at a site. Here each block was a 4–5 m sector of a tree with five traps, each trap 1–2 m apart within the sector (Table 2).

In all tests, traps were hung from branches \sim 1.5–2 m above ground. Traps within blocks were rotated 2 to 18 times (Table 2). Flies were removed from traps every time positions were changed and were saved in cups and later sexed in the laboratory. Traps were replaced after three weeks if needed, with one to three replacements occurring over the 3–8 week tests. Particulars of each test site and its trees follow.

Four five-tree block tests were conducted (Table 2). Two tests were conducted for *R. indifferens*: one in an unmanaged cherry orchard in Yakima with 145 trees \sim 4–5 m tall and wide, and the other in an unmanaged cherry grove in Vancouver with 50 trees \sim 6–8 m tall and \sim 3–5 m wide. Two tests were conducted for *R. pomonella* in an old homestead in Skamania County with approximately 100 apple and 25 black hawthorn trees \sim 5–8 m tall and wide.

Six tests using blocks of one tree were conducted (Table 2). One test for *R. indifferens* was conducted in five seedling cherry trees \sim 5–7 m tall and wide in Roslyn. Three tests for *R. pomonella* were conducted in a contiguous stand of black hawthorn trees \sim 6–8 m tall and wide in the Nile Valley and in individual black hawthorn and apple trees \sim 5–7 m tall and wide in Vancouver. Two tests for *R. completa* were conducted in five walnut trees \sim 3–4 m tall and wide in Zillah, Site 1, and in 12 walnut trees \sim 8–17 m tall and wide in Naches.

Two tests employing multiple blocks per tree were conducted for *R. completa* (Table 2) in English walnut in homeowners' yards. The first test was at Zillah, Site 2, with two trees, each \sim 15–18 m tall and wide; the second was at Donald, with two trees, each \sim 15 m tall and wide.

PL1 + Tanglefoot vs. PA1 and other traps + Tanglefoot. The main purpose of this second set of experiments was to determine if adding or replacing the adhesive already present on a trap with Tanglefoot improves the trap's efficacy. Emphasis was placed on comparing the PL1 + Tanglefoot with the PA1 + Tanglefoot, in most cases with the same sticky surface areas. However, to gain additional information, other yellow traps + Tanglefoot (all baited with ammonium carbonate) were also compared in 2014 and 2015. Blocking schemes and other procedures followed those described for the five-trap comparison tests.

For *R. indifferens*, one test was conducted from 18–29 May 2015 in three sweet cherry trees with one or two blocks per tree for five total blocks in Kennewick, WA. The PL1 + Tanglefoot vs. PA1 + Tanglefoot (each with 596 cm² sticky surface) was the major comparison. The adhesive on the PA1 was scraped off and replaced with Tanglefoot (\sim 5 g each side). To obtain additional information, the traps were compared with three non-rectangle traps. The first was a yellow PALz trap, a plastic rectangle with ends tied together (30.5 \times 22.9 cm, 614 cm² sticky surface; Plant Protection Institute, Budapest, Hungary). This trap was covered with a thick Tanglefoot-like adhesive, so no Tanglefoot was added to it.

Table 2
Tests of five trap types against three *Rhagoletis* species in Washington State, U.S.A. in 2014

Five-Tree Blocks: One Trap in Each Tree						
Fly species	Traps in ^a	Site	No. trees	No. traps per type	Trapping dates	No. trap rotations ^b
<i>R. indifferens</i>	Sweet cherry	Yakima	25	5	19 May–25 June	9
	Sweet cherry	Vancouver	25	5	12 June–14 July	5
<i>R. pomonella</i> ^c	Apple, black hawthorn	Skamania	25	5	4 July–7 Aug	5
	Apple	Skamania	25	5	11 Aug–2 Sept	3
Blocks of One Tree Each: All Five Trap Types in Each Tree						
Fly species	Traps in ^a	Site	No. trees	No. traps per type	Trapping dates	No. trap rotations ^b
<i>R. indifferens</i>	Sweet cherry	Roslyn	5	5	9 July–18 Aug	11
<i>R. pomonella</i>	Black hawthorn	Vancouver	3	3	23 June–16 July	2
	Black hawthorn	Nile Valley	5	5	9 July–3 Sept	13
<i>R. completa</i>	Apple	Vancouver	3	3	3 July–23 July	3
	English walnut	Zillah, Site 1	5	5	2 July–2 Sept	18
	English walnut	Naches	5	5	16 July–29 Aug	12
Multiple Blocks within a Tree, 2 Trees, Each Tree with 2 Blocks, 5 Trap Types Per Block						
Fly species	Traps in ^a	Site	No. trees	No. traps each type	Trapping dates	No. trap rotations ^b
<i>R. completa</i>	English walnut	Zillah, Site 2	2	4	2 July–1 Aug	6
	English walnut	Donald	2	4	30 July–26 Aug	7

^aSweet cherry, *Prunus avium* (L.) L.; black hawthorn, *Crataegus douglasii* Lindley; apple, *Malus domestica* Borkhausen; English walnut, *Juglans regia* (L.).
^bRotated = traps moved over to next position in trees within blocks.
^cThree replicates in apple and two in black hawthorn.

The second trap was a yellow ‘Fly Trap’ with a “modular” design (24.8 cm high \times 8.9 cm wide, 482 cm² sticky surface; PIC Corporation, Linden, NJ), and the third trap was a 9.0-cm diameter yellow ball (283 cm² sticky surface; laboratory made). The Fly Trap had a thin layer of pressure-sensitive adhesive, and the ball had no adhesive; Tanglefoot was added to both. Traps within blocks were rotated four times; due to high fly numbers, traps were replaced each time.

For *R. pomonella* (Table 3), three tests were conducted in 2014 and 2015. All tests used one trap per tree. In Test 1, five blocks of five trees each were set up. In Test 2, five blocks of two trees each were set up. In Test 3, three blocks of two trees each were set up.

For *R. completa* (Table 3), four tests were conducted in 2014 using multiple blocks per tree. In all four *R. completa* tests, five blocks were set up. In Tests 1, 3, and 4, there were two blocks in each of two trees and one block in one. In Test 2, there were two blocks in one tree and three in a second tree.

For both species, traps within blocks were rotated four to seven times, except at Skamania in 2015 (twice; Table 3). In 2014, Tanglefoot was added on top of adhesives already present, but in 2015, the adhesives were scraped off and replaced with Tanglefoot.

Statistics. For each test, fly counts were summed over all collection dates and square-root transformed (Zar 1999; data met normality and homogenous variance assumptions) and then subjected to randomized complete block analysis of variance (ANOVA), followed by Tukey’s HSD test for means separation (SAS Institute Inc. 2010). In a second analysis, counts were adjusted for sticky surface area (cm²) before analysis with ANOVA and Tukey’s HSD test. In addition, orthogonal contrasts were conducted after ANOVA, using the contrast statement in SAS for fly counts per cm² to identify possible common factors affecting captures. Specifically, for the five trap-comparison tests, three contrasts of plastic vs. paper traps or Tanglefoot vs. other adhesives were made: the means of PL1 + PL2 with Tanglefoot vs. means of PA1 + PA2, PA1 + PA3, and PA2 + PA3. For the PL1 + Tanglefoot vs. PA1 and other traps + Tanglefoot tests against *R. pomonella* and *R. completa*, two or four contrasts of Tanglefoot vs. other adhesives were made (none was made for the *R. indifferens* test). Female- and male-fly data were combined to simplify the results, as catch patterns of the sexes were similar.

RESULTS

Five-trap comparisons. Within fly species, relative trap efficacy patterns using five-tree block, one-tree block, and multiple blocks per tree designs were similar, especially for the best performing traps (Figs. 2–4), so the way blocking was performed made no difference in the conclusions. Across all three *Rhagoletis* species, the PL1 and PL2 + Tanglefoot were generally the most efficacious of the five traps tested. Compared with the PL2 + Tanglefoot, the PL1 + Tanglefoot caught statistically more *R. indifferens* in one (Fig. 3A) of three tests, more *R. pomonella* in one (Fig. 3D) of five tests, and more *R. completa* in one (Fig. 4A) of four tests.

The PA3 was the next most efficacious trap, but the PL1 + Tanglefoot caught statistically more flies than the PA3 in nine of twelve tests across species. The PA3 caught statistically more *R. pomonella* than the PA1 in one (Fig. 3D) of five tests and more *R. completa* than the PA1 in three (Figs. 3E, 3F, and 4B) of four tests. In no case did the PA1 catch statistically more flies than the PA3. The PA2 was the least effective of all five traps.

Combining data from all tests, more females than male flies were caught on all trap types. For *R. indifferens*, 58–60% caught on the five trap types were females. For *R. pomonella*, 65–69% of flies caught were females; for *R. completa*, 55–66% were females.

Table 3
Tests of PL1 + Tanglefoot vs. PA1 + Tanglefoot and other traps against *Rhagoletis pomonella* and *R. completa* in Washington State, U.S.A., in 2014 and 2015. Numbers of traps of each type tested are shown inside the table.

<i>R. pomonella</i> : One Trap per Apple Tree					
Trap Type	Sticky surface area, cm ²	Test 1: Five blocks of five trees each; Skamania, 4 Sept–16 Oct 2014	Test 2: Five blocks of two trees each; Skamania, 3–29 Sept 2015	Test 3: Three blocks of two trees each; Roslyn, 17 Aug–24 Sept 2015	
PL1	596	5	—	—	
PL1 + Tanglefoot	596	5	5	3	
PA1 + Tanglefoot	596	5	5	3	
PA2 + Tanglefoot	596	5	—	—	
No. trap rotations		7	2	6	
<i>Rhagoletis completa</i> : Multiple Blocks per Walnut Tree					
Trap Type	Sticky surface area, cm ²	Test 1: Three trees, each with one block or two blocks; Naches, 27 Aug–26 Sept 2014	Test 2: Two trees, one with three blocks and one with two blocks; Donald, 27 Aug–26 Sept 2014	Test 3: Three trees, each with one block or two blocks; Naches, 29 Aug–26 Sept 2014	Test 4: Three trees, each with one block or two blocks; Naches, 29 Aug–26 Sept 2014
PL1	596	5	5	5	—
PL1 + Tanglefoot	596	5	5	5	—
PA1	407	5	—	—	—
PA1 + Tanglefoot	407	5	5	—	—
PA1 + Tanglefoot	596	—	5	—	—
PA2 + Tanglefoot	596	—	—	5	—
PA1	478	—	—	—	5
PA1 + Tanglefoot	478	—	—	—	5
PA3 + Tanglefoot	478	—	—	—	5
No. trap rotations		6	6	5	4
—: trap not tested.					

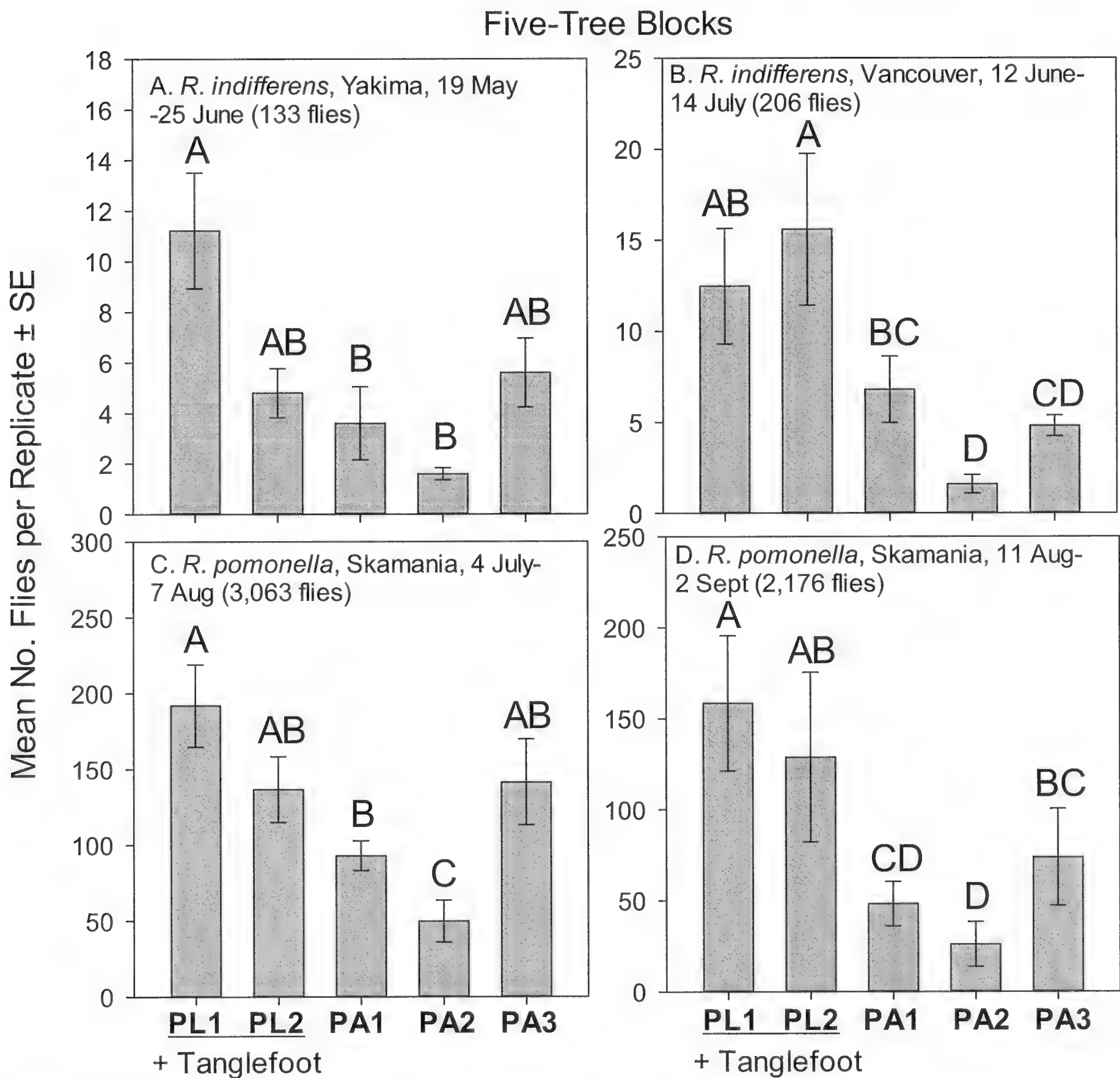


Figure 2. Five-tree block tests: mean numbers of flies (sexes combined) caught per replicate \pm SE in 2014: (A) *R. indifferens* in Yakima; (B) *R. indifferens* in Vancouver; (C) *R. pomonella* in Skamania in apple and hawthorn; (D) *R. pomonella* in Skamania in apple. (A) $F = 5.96$; $df = 4, 16$; $P = 0.0039$; (B) $F = 17.70$; $df = 4, 16$; $P < 0.0001$; (C) $F = 16.77$; $df = 4, 16$; $P < 0.0001$; (D) $F = 19.34$; $df = 4, 16$; $P < 0.0001$. Means within tests with same letters are not significantly different (Tukey’s HSD test, $P > 0.05$).

PL1 + Tanglefoot vs. PA1 and other traps + Tanglefoot. The PL1 + Tanglefoot caught statistically more *R. indifferens* than the PA1 + Tanglefoot when both had 596 cm² sticky surfaces (Fig. 5). It also caught more flies than two non-rectangle traps with Tanglefoot, although statistically not more than the PALz (Fig. 5), which had a different adhesive. However, unlike for *R. indifferens*, mean catches of *R. pomonella* on the PL1 + Tanglefoot and PA1 + Tanglefoot with 596 cm² sticky surfaces did not differ statistically in three tests (Figs. 6A–6C). Similarly to those of *R. pomonella*, mean catches of *R. completa* on the PL1 + Tanglefoot with a 596 cm² sticky surface and PA1 + Tanglefoot with 407 and 596 cm² sticky surfaces did not differ (Figs. 7A and 7B). However, the PL1 + Tanglefoot caught more *R. pomonella* than the PA2 + Tanglefoot (Fig. 6A) and more *R. completa* than both the PA2 + Tanglefoot (Fig. 7C) and PA3 + Tanglefoot (Fig. 7D) when sticky surface areas were equal.

Fly captures adjusted for sticky surface area. In the first set of the five-trap comparisons, the relative efficacies of traps based on catch numbers not adjusted and adjusted for sticky surface area differed, but the major patterns were the same (Table 4).

Notably, for *R. indifferens*, the PL1 + Tanglefoot was still more efficacious than the PA2 and PA3 in two of three tests; for *R. pomonella*, the PL1 + Tanglefoot was more efficacious than the PA1 in three of five tests, and the PL1 and PL2 + Tanglefoot were more efficacious than the PA2 in all five tests; the PL1 + Tanglefoot was more so than the PA3 in four of five tests. For *R. completa*, the PL1 + Tanglefoot was more efficacious than the PA1 and PA2 in all four tests—more so than the PA3 in two of four tests (Table 4).

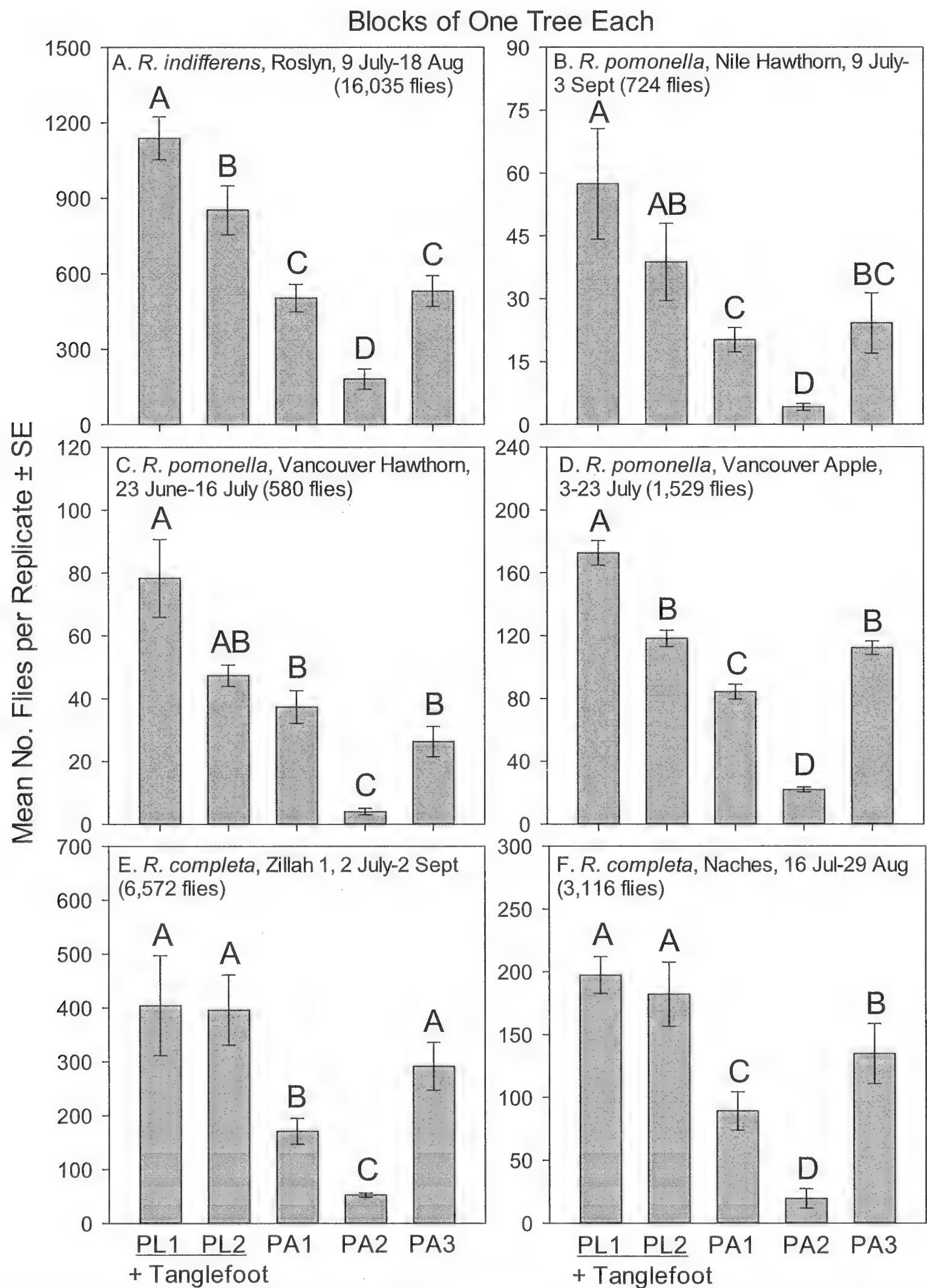


Figure 3. Blocks of one-tree tests: mean numbers of flies (sexes combined) caught per replicate ± SE in 2014: (A) *R. indifferens* in Roslyn; (B) *R. pomonella* in Nile; (C) *R. pomonella* in Vancouver in hawthorn; (D) *R. pomonella* in Vancouver in apple; (E) *R. completa* in Zillah, Site 1; (F) *R. completa* in Naches. (A) $F = 116.27$; $df = 4, 16$; $P < 0.0001$; (B) $F = 33.51$; $df = 4, 16$; $P < 0.0001$; (C) $F = 1,631.26$; $df = 4, 8$; $P < 0.0001$; (D) $F = 47.77$; $df = 4, 16$; $P < 0.0001$; (E) $F = 90.56$; $df = 4, 16$; $P < 0.0001$; (F) $F = 90.56$; $df = 4, 16$; $P < 0.0001$. Means within tests with same letters are not significantly different (Tukey's HSD test, $P > 0.05$).

Orthogonal contrasts. For the first set of the five-trap comparisons, contrasts between the means of PL1 + PL2 with Tanglefoot vs. means of PA1 + PA2, PA1 + PA3, and PA2 + PA3 differed regardless of fly species (Table 5). This suggests plastic material or Tanglefoot contributed to higher fly catches. In the PL1 + Tanglefoot and other traps + Tanglefoot comparisons for *R. pomonella*, results suggested Tanglefoot increased captures on PL1, PA2, and PA3, but not on PA1 (Table 5); for *R. completa*, results suggest Tanglefoot increased captures on PL1 and PA1 (Table 5).

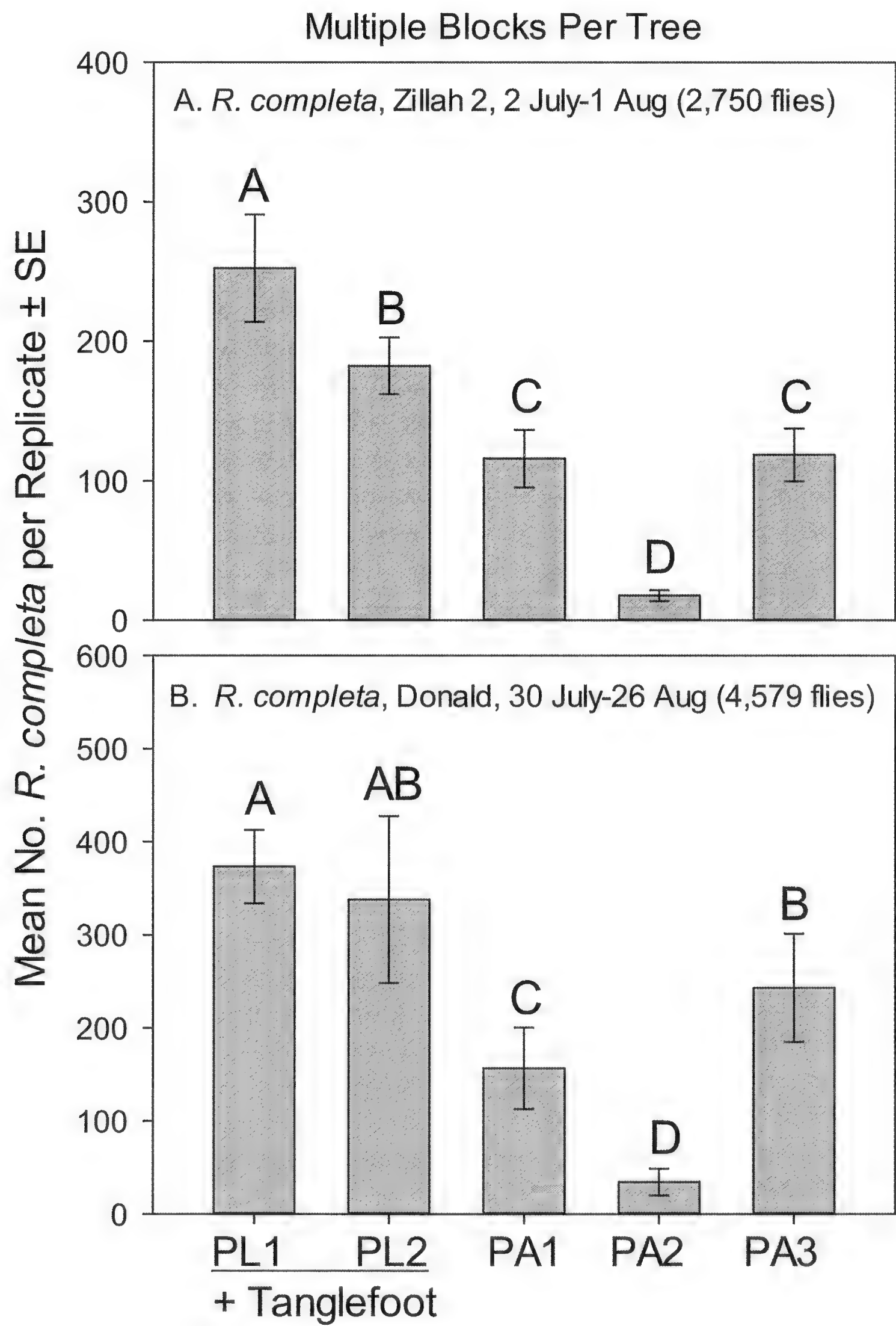


Figure 4. Multiple blocks per tree tests: mean numbers of flies (sexes combined) caught per replicate ± SE in 2014: (A) *R. completa* in Zillah, Site 2; (B) *R. completa* in Donald. (A) $F = 88.45$; $df = 4, 12$; $P < 0.0001$; (B) $F = 80.80$; $df = 4, 12$; $P < 0.0001$. Means within tests with the same letters are not significantly different (Tukey’s HSD test, $P > 0.05$).

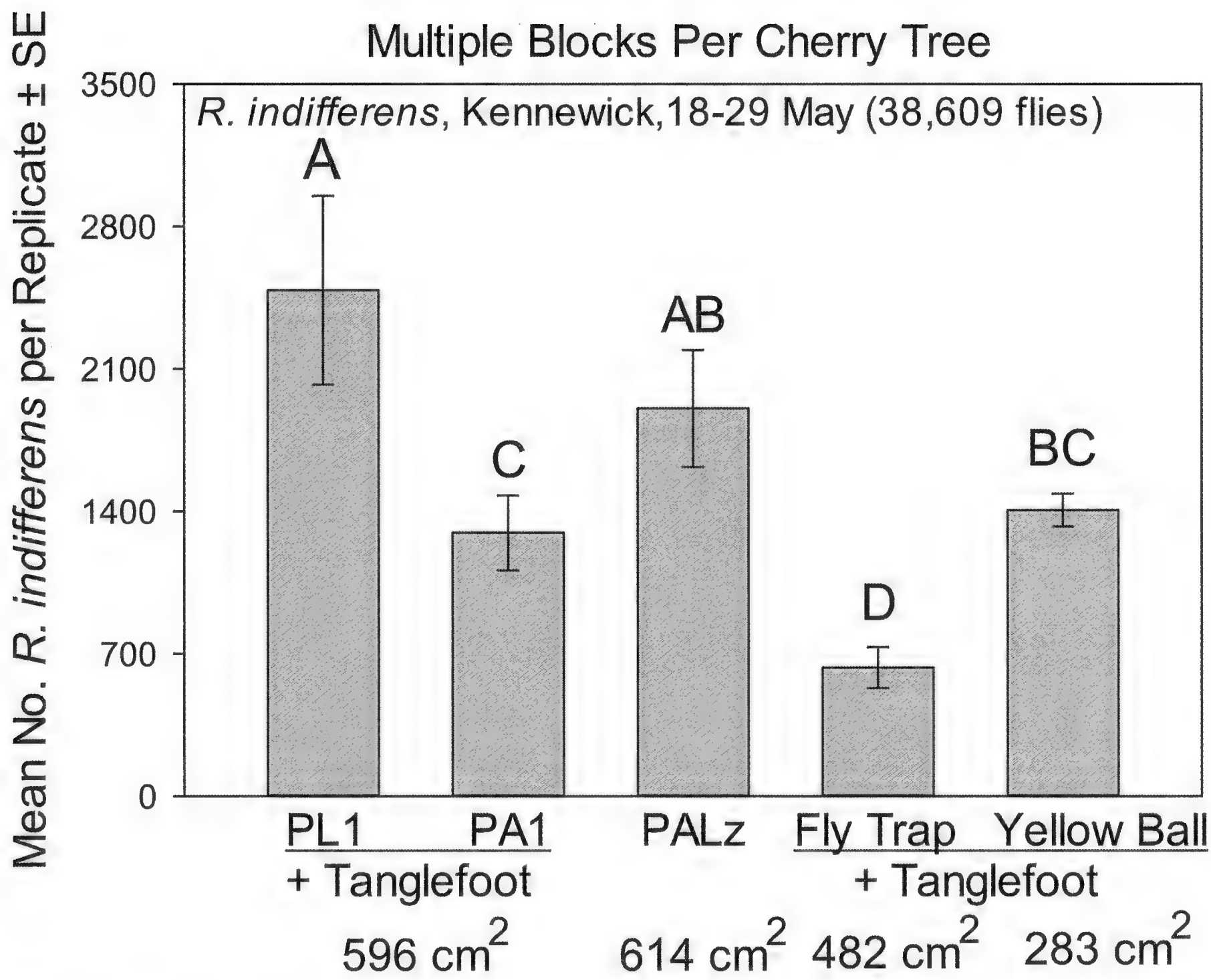


Figure 5. Mean captures of *Rhagoletis indifferens* (sexes combined) ± SE per replicate on PL1 + Tanglefoot vs. PA1 + Tanglefoot and other traps in Kennewick in 2015. Sticky surface areas are shown below trap types. $F = 31.27$; $df = 4, 16$; $P < 0.001$. Means with same letters are not significantly different (Tukey's HSD test, $P > 0.05$).

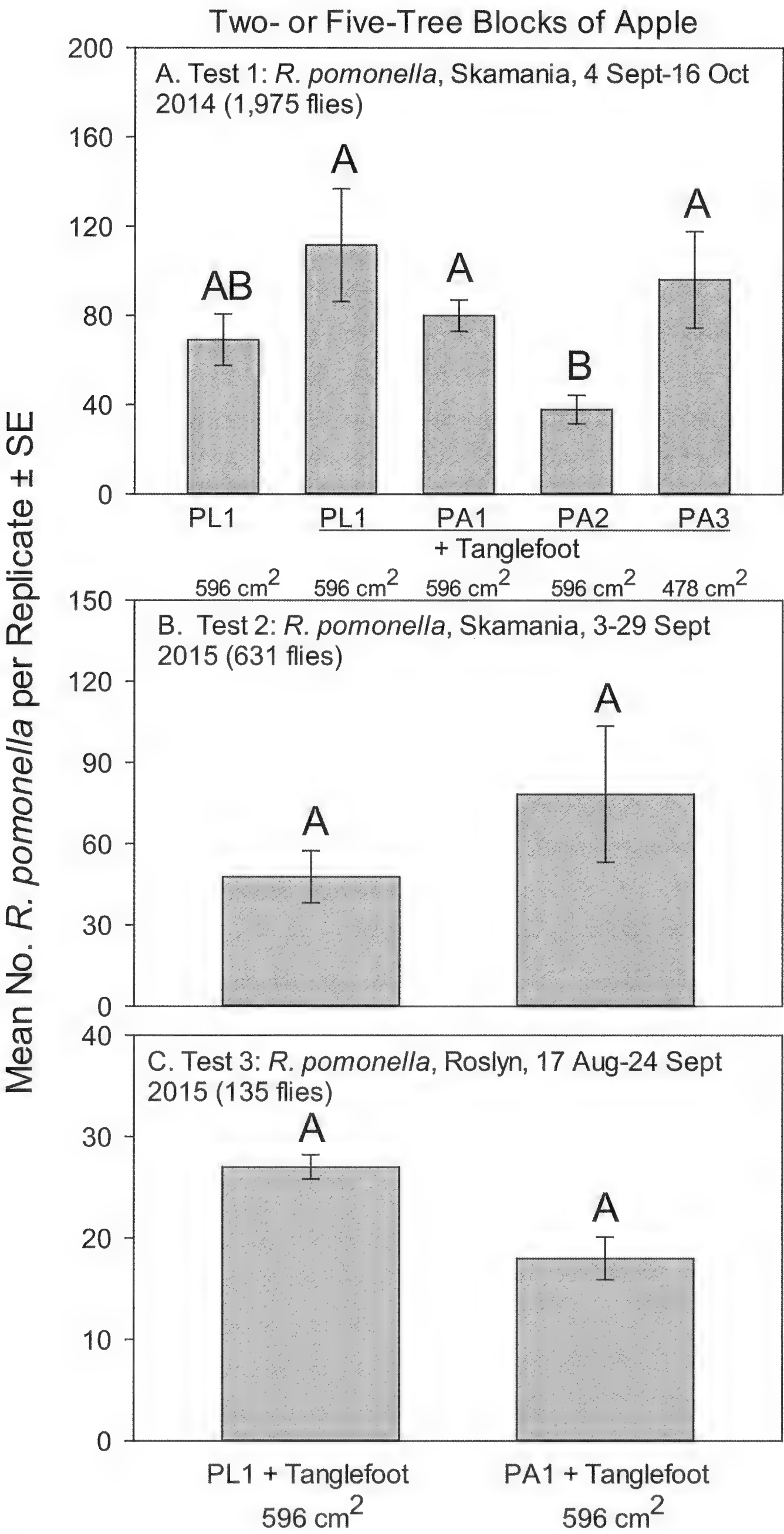


Figure 6. Mean captures of *Rhagoletis pomonella* (sexes combined) ± SE per replicate in 2014 and 2015 on (A) PL1 and PL1 + Tanglefoot vs. PA1 + Tanglefoot, PA2 + Tanglefoot, and PA3 + Tanglefoot; PL1 + Tanglefoot vs. PA1 + Tanglefoot in (B) Skamania and in (C) Roslyn. Sticky surface areas are shown below trap types. (A) $F = 7.10$; $df = 4, 16$; $P = 0.0017$; (B) $F = 1.66$; $df = 1, 4$; $P = 0.2669$; (C) $F = 8.13$; $df = 1, 2$; $P = 0.1041$. Means within tests with same letters are not significantly different (Tukey's HSD test, $P > 0.05$).

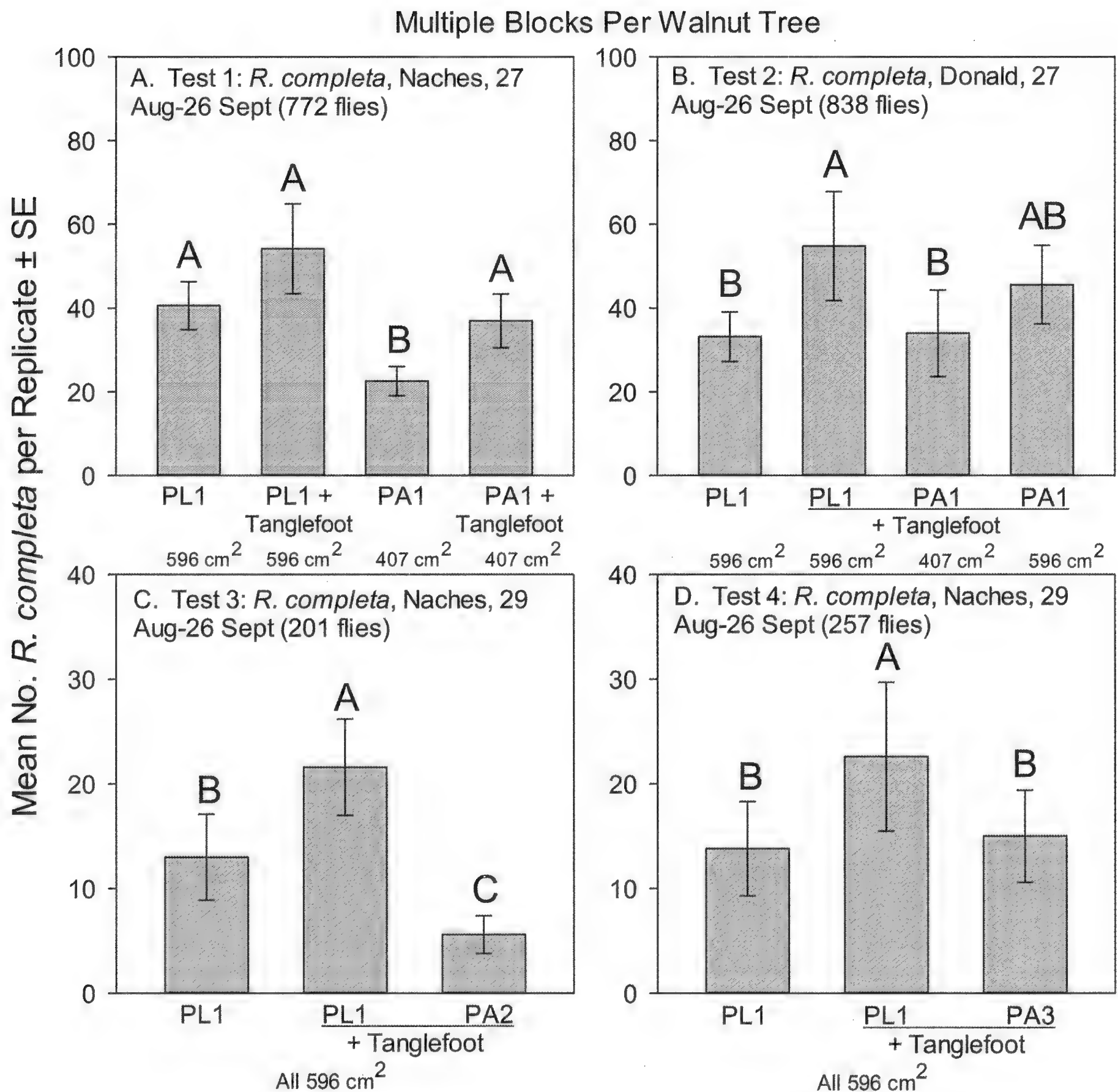


Figure 7. Mean captures of *Rhagoletis completa* (sexes combined) \pm SE per replicate in 2014 on PL1 and PL1 + Tanglefoot vs. (A, B) PA1 and PA1 + Tanglefoot; (C) PA2 + Tanglefoot; and (D) PA3 + Tanglefoot. Sticky surface areas are shown below trap types. (A) $F = 12.35$; $df = 3, 12$; $P = 0.0006$; (B) $F = 8.64$; $df = 3, 12$; $P = 0.0025$; (C) $F = 19.34$; $df = 2, 8$; $P = 0.0009$; (D) $F = 7.63$; $df = 2, 8$; $P = 0.0140$. Means within tests with same letters are not significantly different (Tukey's HSD test, $P > 0.05$).

Table 4
Comparisons of results of Tukey's HSD tests on mean numbers of *Rhagoletis* flies caught on traps unadjusted vs. adjusted for sticky surface area. Letters in front of species indicate different tests. Values in front of adjusted letters are mean flies/cm². Means for unadjusted catches are shown in Figs. 2-7. Same letters within columns indicate means of trap treatments are not significantly different ($P > 0.05$).

Five-Trap Comparisons: Five-Tree Blocks									
Trap Type	A. <i>R. indifferens</i>		B. <i>R. indifferens</i>		C. <i>R. pomonella</i>		D. <i>R. pomonella</i>		
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	
PL1 + Tanglefoot	A	0.018A	AB	0.021A	A	0.322A	A	0.266A	
PL2 + Tanglefoot	AB	0.008A	A	0.026A	AB	0.229A	AB	0.216AB	
PA1	B	0.009A	BC	0.017AB	B	0.228A	CD	0.199CD	
PA2	B	0.005A	D	0.005C	C	0.159B	D	0.083D	
PA3	AB	0.012A	CD	0.010B	AB	0.296A	BC	0.154BC	
Five-Trap Comparisons: Blocks of One Tree Each									
Trap	A. <i>R. indifferens</i>		B. <i>R. pomonella</i>		C. <i>R. pomonella</i>				
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	
PL1 + Tanglefoot	A	1.911A	A	0.096A	A	0.131A			
PL2 + Tanglefoot	B	1.430B	AB	0.065B	AB	0.080BC			
PA1	C	1.236B	C	0.050B	B	0.091AB			
PA2	D	0.580C	D	0.013C	C	0.013D			
PA3	C	1.110B	BC	0.051B	B	0.055C			
	D. <i>R. pomonella</i>		E. <i>R. completa</i>		F. <i>R. completa</i>				
Trap	Unadjusted		Unadjusted		Unadjusted		Unadjusted		
PL1 + Tanglefoot	A	0.289A	A	0.678A	A	0.331A			
PL2 + Tanglefoot	B	0.199C	A	0.664A	A	0.306A			
PA1	C	0.207C	B	0.419B	C	0.219B			

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PA2	D	0.070D	C	0.167C	D	0.063C
PA3	B	0.235B	A	0.610A	B	0.282A
Five-Trap Comparisons: Multiple Blocks Per Tree						
			A. R. completa			
Trap		Unadjusted	Adjusted	Unadjusted	Adjusted	
PL1 + Tanglefoot		A	0.215A	A	0.626A	
PL2 + Tanglefoot		B	0.134AB	AB	0.567AB	
PA1		C	0.130B	C	0.385C	
PA2		D	0.026C	D	0.110D	
PA3		C	0.100B	B	0.508B	
PL1 + Tanglefoot vs. PA1 and other traps + Tanglefoot comparisons						
			B. R. pomonella			
Trap		Unadjusted	Adjusted	Unadjusted	Adjusted	
PL1 + Tanglefoot	A	4.172AB		AB	0.116B	
PA1 + Tanglefoot	C	2.170C		A	0.187A	
PALz	AB	3.105B		A	0.134AB	
Fly Trap + Tanglefoot	D	1.309D		B	0.064C	
Yellow Ball + Tanglefoot	BC	4.965A		A	0.201A	
PL1 + Tanglefoot vs. PA1 and other traps + Tanglefoot comparisons						
			D. R. completa			
Trap		Unadjusted	Adjusted	Unadjusted	Adjusted	
PL1	A	0.068AB		B	0.056B	
PL1 + Tanglefoot	A	0.091A		A	0.092A	
PA1	B	0.055B		B	0.083A	
PA1 + Tanglefoot	A	0.091A		AB	0.077A	

Table 5

Orthogonal contrasts of *Rhagoletis* fly catches adjusted for sticky surface area on traps in a randomized block ANOVA. Letters before the species indicate different tests. Traps with higher fly catches are in bold.

Five-Trap Comparisons: Five-Tree Blocks									
Contrasts	A. R. indifferens		B. R. indifferens		C. R. pomonella		D. R. pomonella		
	F	P	F	P	F	P	F	P	
PL1 + PL2 + Tanglefoot vs. PA1 + PA2	5.77	0.0288	25.85	0.0001	8.48	0.0102	36.14	<0.0001	
PL1 + PL2 + Tanglefoot vs. PA1 + PA3	1.41	0.2531	11.14	0.0042	0.14	0.7092	15.60	0.0011	
PL1 + PL2 + Tanglefoot vs. PA2 + PA3 ^a	2.41	0.1401	39.44	<0.0001	3.77	0.0698	28.51	<0.0001	
Five-Trap Comparisons: Blocks of One Tree Each									
Contrasts	A. R. indifferens		B. R. pomonella		C. R. pomonella				
	F	P	F	P	F	P	F	P	
PL1 + PL2 + Tanglefoot vs. PA1 + PA2	112.40	<0.0001	56.25	<0.0001	35.85	<0.0001	0.0003		
PL1 + PL2 + Tanglefoot vs. PA1 + PA3	37.61	<0.0001	15.66	0.0011	8.07	0.0218			
PL1 + PL2 + Tanglefoot vs. PA2 + PA3	131.68	<0.0001	58.60	<0.0001	60.38	<0.0001			
Contrasts	D. R. pomonella		E. R. completa		F. R. completa				
	F	P	F	P	F	P	F	P	
PL1 + PL2 + Tanglefoot vs. PA1 + PA2	1,790.44	<0.0001	73.89	<0.0001	117.84	<0.0001			
PL1 + PL2 + Tanglefoot vs. PA1 + PA3	49.02	0.0001	3.03	0.1009	12.19	0.0030			
PL1 + PL2 + Tanglefoot vs. PA2 + PA3	1,410.31	<0.0001	62.78	<0.0001	86.57	<0.0001			

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Five-Trap Comparisons: Multiple Blocks Per Tree

Contrasts	A. R. completa		B. R. completa			
	F	P	F	P		
PL1 + PL2 + Tanglefoot vs. PA1 + PA2	24.88	0.0003	148.62	<0.0001		
PL1 + PL2 + Tanglefoot vs. PA1 + PA3	7.86	0.0160	19.62	0.0008		
PL1 + PL2 + Tanglefoot vs. PA2 + PA3	35.30	<0.0001	105.78	<0.0001		
PL1 + Tanglefoot vs. PA1 and other traps + Tanglefoot comparisons						
Contrasts	A. R. pomonella		B. R. completa		C. R. completa	
	F	P	F	P	F	P
PL1 vs. PL1 + Tanglefoot ^b	5.53	0.0318	4.46	0.0563	12.90	0.0037
PL1 vs. PA1 + Tanglefoot ^c	0.65	0.4314	5.03	0.0446	5.01	0.0449
PL1 vs. PA2 + Tanglefoot	5.66	0.0302	---	---	---	---
PL1 vs. PA3 + Tanglefoot	8.02	0.0120	---	---	---	---

^aExcept for A. R. indifferens. ^bExcept for B. R. completa. ^cExcept for A. R. pomonella.

DISCUSSION

The plastic rectangles supplemented with Tanglefoot were the most efficacious of the five sticky yellow rectangle traps tested against *R. indifferens*, *R. pomonella*, and *R. completa*. Until recently (Yee 2011, 2014), the PA1 (Pherocon AM) could be assumed to be the most efficacious yellow rectangle against most *Rhagoletis* flies in North America. However, the PA1 had usually been compared with spheres and Ladd traps and not with other yellow rectangles against *R. pomonella* and *R. completa*, as well as the blueberry maggot, *R. mendax* Curran (e.g., Prokopy and Hauschild 1979; AliNiazee *et al.* 1987; Riedl *et al.* 1989; Liburd *et al.* 2001; Teixeira and Polavarapu 2001). In the current study, the PA1 was only more efficacious than the PA2, as it was against *R. cerasi* (Katsoyannos *et al.* 2000). It is unclear whether traits of the PA1 have changed over the years. For this reason, the trap traits documented in Fig. 1 and Table 1 can be useful for future comparisons of traps tested here with other, newer traps.

Based on their superior performance against high *Rhagoletis* populations, the plastic traps + Tanglefoot may be able to detect lower fly populations than all the cardboard traps tested here, perhaps making them better options for monitoring. Plastic and cardboard traps cost about the same (~U.S.\$1.10 per trap; cost of 10 g TF per trap is ~9 cents), but have the advantage of not fading over the season, as can the PA2 and PA3 (W. L. Y., personal observations). There are, however, several disadvantages to the plastic traps. One is that they are thinner and lighter, so are more prone to flap in heavy winds. This sometimes causes them to tear loose from branches; however, this can be prevented by securing the traps to branches using three ties instead of one. Another disadvantage is that, as currently manufactured, plastic traps would need to be supplemented with Tanglefoot. Thus, caution should be taken when deciding which traps to use, because overall trap catch is not always necessarily the deciding factor in selecting an ‘optimal’ trap for monitoring purposes. Traps need only be effective enough to provide a consistent, reliable ‘sample’ or estimate of a population, with minimal cost and time in servicing.

All traps generally caught more females than males, consistent with findings for *R. mendax* (e.g., Liburd *et al.* 2001; Teixeira and Polavarapu 2001). Other studies showed that 46%, 57%, and 50% of *R. indifferens*, *R. pomonella*, and *R. completa* that emerged from soil under cages, respectively, were females (Frick *et al.* 1954; Dean and Chapman 1973; Boyce 1934). Because females comprised 55–69% of trap catches here, the traps may be slightly biased towards females, suggesting males may be less attracted to yellow rectangles than females.

The major objective here was to identify the most efficacious of five commercial sticky yellow rectangles against flies, but a secondary objective was to determine which factors might affect their efficacies. Differences in sticky surface areas, color, translucence, and adhesive type make identifying factors responsible for the greater efficacies of the PL1 and PL2 + Tanglefoot vs. the PA1, PA2, and PA3 (Figs. 2–4) difficult, but there are at least three possible factors. One is that the sticky surface areas of the plastic traps were larger; however, analyses of catches per sticky area suggest this was of minimal importance for all three species. A second possible factor is that all three fly species were most stimulated visually by color and other cues in the plastic traps. The third possible factor is that supplementing the plastic traps with Tanglefoot increased their efficacy either by making them tackier due to composition or amount or by altering their visual properties.

Results comparing the PL1 and PA1 both with Tanglefoot and with equal sticky surface areas against *R. indifferens* in 2015 suggest that Tanglefoot did not cause the greater efficacy of the PL1 + Tanglefoot vs. the PA1 against *R. indifferens* in 2014 tests. More likely, the yellow color and translucence of the PL1 caused this (Yee 2014). The PL1 + Tanglefoot was also better than two non-rectangle traps with Tanglefoot for catching high numbers of *R. indifferens*, further suggesting traits of the PL1 itself

independent of Tanglefoot were responsible for its high efficacy. Perhaps not coincidentally, the PaLz trap was plastic and also performed well.

In contrast to results for *R. indifferens*, Tanglefoot on the PL1 appeared responsible for the higher efficacy of the PL1 + Tanglefoot vs. the PA1 against *R. pomonella* and *R. completa*, based on tests where sticky surface areas were equal. Tanglefoot may be tackier and/or had a lower viscosity than the adhesive on the PA1, making flies stick faster. Less likely, it increased the visual attractiveness of the trap. Polybutene is the active ingredient in Tanglefoot (Contech 2014) and presumably also in the adhesive on the PA1 (exact chemical compositions of the adhesives are unpublished). Even though both adhesives are clear or slightly cloudy, particular polymers, grade, or amount of polybutene in Tanglefoot and other adhesives probably differ.

Supplementing the PA2 and PA3 with Tanglefoot resulted in variable outcomes for *R. pomonella* and *R. completa*. For both flies, Tanglefoot was not a factor in why the PL1 + Tanglefoot performed better than the PA2. The distinct reflectance/color of the PA2 (Fig. 1F) may simply have been less attractive. In contrast, for *R. pomonella*, the PL1 + Tanglefoot appeared better than the PA3 solely because of the Tanglefoot. Traits of the PA3, PL1, and PA1 thus may be similarly attractive to *R. pomonella*. However, for *R. completa*, the PL1 + Tanglefoot caught more flies than the PA3 + Tanglefoot, so traits of the PL1 may have been more attractive for this species, although more tests are needed to confirm this.

In summary, results suggest the plastic rectangles + Tanglefoot tested here are better than standard cardboard rectangles for capturing high numbers of all three *Rhagoletis* species. This implies these should be the rectangles of choice for monitoring these species. The efficacy of some cardboard rectangles tested against *R. pomonella* and *R. completa* but not against *R. indifferens* may be increased simply by using an alternative or more adhesive. This suggests similar trap efficacies against the three species may have different underlying causes, which if true has implications for the development of more species-specific and efficacious traps.

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SCIENTIFIC NOTE

**Pacific Flatheaded Borer, *Chrysobothris mali* Horn
(Coleoptera: Buprestidae), found attacking apple saplings
in the Southern Interior of British Columbia**

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Key words: *Chrysobothris mali*, Pacific flatheaded borer, *Malus domestica*

The Pacific flatheaded borer, *Chrysobothris mali* Horn (Coleoptera: Buprestidae), is widely distributed throughout western North America, occurring west of the Rocky Mountains from California to British Columbia and western provinces (Furness and Carolin 1977; Solomon 1995). It feeds on 41 genera of plants in 20 families, including *Malus* (Burke 1929). We investigated borer damage to young apple, *Malus domestica*, saplings reported by a grower in July 2015 in Kelowna, British Columbia. The saplings were planted in April 2014 and 2015. Larvae were actively feeding on B9 and M9 rootstocks imported from the Netherlands and grafted with gala and honeycrisp apple varieties. The report was a concern, because flatheaded borer is not a pest of apples in British Columbia. Apple clearwing moth, a serious pest of apples in British Columbia was imported on rootstock from the Netherlands. The study was conducted to identify the *Chrysobothris* species, its distribution and infestation levels to help guide management recommendations.

Infested trees had dark-coloured, cracked bark with frass showing through. Frass was evident around the base of some infested trees. Larvae were found beneath the bark and inside the wood, and larval galleries were exposed when bark was removed; one larva was found in a sapling, rarely two in a sapling. The galleries occasionally encircled the stem, which killed the sapling. Leaf symptoms on infested saplings varied from yellow to purple.

Surveys were conducted in 19 young apple plantings and nurseries in the Okanagan and Similkameen valleys of British Columbia, from July to October 2015, to determine distribution and infestation levels. All saplings at each survey site were visually inspected for damage and presence of larvae (Table 1). Survey sites were located in Kelowna, Oyama, Winfield, Lavington, Cawston, and Keremeos. Site selections were based on information about young apple plantings provided by B.C. Tree Fruits Ltd. We inspected infested saplings at the original site, but it was not included in our surveys, because the grower had removed some of the infested trees.

To confirm whether the borer was a native or imported *Chrysobothris* species, 20 infested saplings, including six from the original site, were sent to the Canadian Food Inspection Agency (CFIA) Entomology laboratory in Ottawa for larval and adult identifications. Infested saplings were also kept in the B.C. Ministry of Agriculture

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Laboratory in Kelowna for rearing out adults. Four larvae were sent to Robert Foottit (AAFC) for DNA barcoding.

Two of four larval specimens were barcode sequenced, producing identical sequences, and matched to *C. mali*. Species identifications were based on sequencing of the DNA barcode region of the mitochondrial gene Cytochrome C oxidase subunit I (COI). DNA was extracted, amplified, and sequenced according to standard DNA barcode protocols (http://www.barcodeoflife.org/sites/default/files/Protocols_for_High_Volume_DNA_Barcode_Analysis.pdf), then compared to reference sequences in BOLD (Barcode of Life Data Systems (<http://www.boldsystems.org>), based on specimens in the Canadian National Collection, collected in Alberta, Saskatchewan and California. The match to *C. mali* ranged from 99.5 to 98.8 percent, whereas the similarity to 22 other species ranged from 82.0 to 95.0 percent. Sequences were deposited in GenBank, National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, MD, USA (<http://www.ncbi.nlm.nih.gov/genbank/>; GenBank Accession No. KX283168).

The CFIA and the B.C. Ministry of Agriculture laboratories reared out adult *C. mali*. *Chrysobothris mali* larvae were detected at low infestation levels (0.01 to 0.22%) in 15 of the 19 survey sites (Table 1).

Table 1
Flatheaded borer larval infestation levels in young apple orchards/nurseries in the Okanagan and Similkameen valleys of British Columbia in 2015.

Orchard/Nursery	Number of trees examined	Number of infested trees	Infestation Level (%)
A	42,000	10	0.02
B	14,000	1	0.01
C	14,000	1	0.01
D	90,000	45	0.05
E	15,000	33	0.22
F	10,000	0	0
G	17,500	21	0.12
H	35,000	11	0.03
I	55,000	10	0.02
J	4,000	0	0
K	150,000	147	0.10
L	40,000	0	0
M	15,000	4	0.02
N	245,000	1	0
O	5,000	1	0.02

There were previous unconfirmed reports of *C. mali* damage to young apple trees in Kelowna in 2003 (Philip 2003). Our study provides the first confirmed record of *C. mali* causing damage on young fruit trees in the Okanagan. *Chrysobothris mali* has previously been reported as a pest of newly planted fruit trees and of young nursery trees in California (Burke 1919; Burke 1929; McNelly *et al.* 1969). McNelly *et al.* (1969) reported that young trees stressed by sunburn, drought, or bark injury, or planted too late in the spring were particularly subject to attack.

Chrysobothris mali produces a single generation per year. It overwinters as mature larvae in the heartwood. Pupation occurs between April and May. Adult emergence and oviposition occurs in June and July (Burke 1929). Eggs are laid in cracks and crevices in the bark. Eggs hatch, and larvae mine into the cambium and pack frass in the mine behind it (Davis *et al.* 1968).

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We thank Caitlin Miller, Diane Thomas, Hugh Philip, Carl Withler, and B.C. Tree Fruits Ltd. staff for help with surveys, and participating apple nurseries, rootstock importers and growers for access to survey sites. We thank Amanda Biernacka-Larocque (AAFC, Ottawa) for carrying out the barcode sequencing, and Eduard Jendek (CFIA, Ottawa) for larvae and adult identifications. Funding for the survey was provided by the B.C. Fruit Growers' Association.

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NATURAL HISTORY AND OBSERVATIONS

**First Canadian records for two invasive seed-feeding bugs,
Arocatus melanocephalus (Fabricius, 1798) and *Raglius
 alboacuminatus* (Goeze, 1778), and a range extension for a
 third species, *Rhyparochromus vulgaris* (Schilling, 1829)
 (Hemiptera: Heteroptera)**

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 EMMA J. WALKER⁶, AND J. ROBERTS⁷**

New invasive insect species affect agriculture, the environment, landscapes, and homeowners. Invasive species are difficult and challenging to manage due to limited availability of direct control products or other management strategies. Nuisance insect outbreaks can have negative impacts on homeowners and businesses; if they reach high densities, they can cause anxiety and discomfort and additional management costs. We report here the first records from Canada for two new invasive pests, *Arocatus melanocephalus* and *Raglius alboacuminatus*, and provide a range extension for *Rhyparochromus vulgaris* (Schilling), reported as new to British Columbia by Scudder (in press, this volume).

The elm seed bug, *Arocatus melanocephalus* Fabricius (Hemiptera: Heteroptera: Lygaeidae), is native to Europe and widely distributed in central and southern Europe (Ferracini and Alma 2008); it was reported in China in 2013 (Gao *et al.* 2013). *Arocatus melanocephalus* was first detected in the United States, in Idaho, in 2009 and is present in Oregon, Washington, and Utah (Collman and Bush 2016). An infestation of *A. melanocephalus* was reported by a homeowner in the Rutland area of Kelowna, British Columbia, in June 2016. There were large numbers of adults in and around the home and on Chinese elm wood piles in the yard. Three homeowners in the area also reported *A. melanocephalus* outbreaks.

Adults are 6.5–7 mm long, strongly punctate, with conspicuously contrasting dark-red and black coloration—black on head, posterior lobe of pronotum, scutellum, and posterior half of corium, and rusty red on anterior lobe of pronotum, outer portion of clavus, and anterior portion of corium (Gao *et al.* 2013; figures 1a and 1b).

The life cycle of *A. melanocephalus* in Canada has not been determined. In Europe and the United States, there is one generation per year (Maistrello *et al.* 2006; Idaho State Department of Agriculture 2013). They overwinter as adults and emerge in the spring to lay eggs on elm. Nymphs feed on elm seeds from May to June and develop into adults in the summer (Idaho State Department of Agriculture 2013). *Arocatus melanocephalus*

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feed primarily on elm seeds (*Ulmus* spp.), but have been collected from oak (*Quercus*) and linden (*Tilia*) (Bechinski *et al.* 2012).



Figure 1a. Dorsal view of adult *Arocatus melanocephalus*. Photograph by W. B. Strong



Figure 1b. Lateral view of adult *Arocatus melanocephalus*. Photograph by W. B. Strong

An outbreak of *Raglius alboacuminatus* (Hemiptera: Heteroptera: Rhyparochromidae) was reported by an orchardist in Kelowna, British Columbia, in August 2016. The source of the infestation appeared to be a cut hayfield next to the

property. There were large numbers of *R. alboacuminatus* at the grower's fruit shop, apple bins, and in and around the home and other buildings and structures on the property.

Raglius alboacuminatus is native to Europe and the Mediterranean. It was first detected in North America, in Utah, in 1999, and it is present in California, Oregon, and Washington (Henry 2004; Bechinski and Merickel 2007). Adults are about 5–6 mm long, with dark-brown to black coloration, white markings on the posterior pronotal lobe, anterior half of the corium, and three conspicuous white spots—one on the apex of each corium, and one on the apex of hemelytral membrane (figures 2a and 2b).



Figure 2a. Dorsal view of *Raglius alboacuminatus*. Photograph by W. B. Strong



Figure 2b. Lateral view of *Raglius alboacuminatus*. Photograph by W. B. Strong

The life cycle of *R. alboacuminatus* in Canada has not been determined. There is one generation per year in the United States (Bechinski and Merickel 2007), but there are two in England and three in Russia (Henry 2004; Southwood and Leston 1959). *Raglius alboacuminatus* overwinters as adult. Overwintered adults lay eggs in the soil or ground litter in early spring. Nymphs feed on developing seeds of plants in the mint family (Lamiaceae). The preferred host in the U.K. is black horehound, *Ballota nigra* (Bantock and Botting 2013).

In addition to the two species new to British Columbia, we also report the occurrence of a third species of the exotic seed bug, *Rhyparochromus vulgaris* (Schilling) (Hemiptera: Heteroptera: Rhyparochromidae), from two locations in Kelowna. It was collected in conjunction with *A. melanocephalus* in September 2016 and as by-catch in surveillance traps for invasive alien species from the Kelowna landfill (49.948597° N, 119.424989° W) in June 2016. The initial discovery of *R. vulgaris* in British Columbia is reported by Scudder (in press). All three species are the first representatives of these genera in Canada.

Although *A. melanocephalus* and *R. alboacuminatus* are not known as agricultural pests, the presence of large numbers could be problematic for homeowners and farmers. Elm is a common landscape tree in Interior British Columbia, which may lead to more reports of infestations by *A. melanocephalus*. Years with high summer temperatures may see particularly large numbers, as was found in Italy (Maistrello *et al.* 2006). The large numbers of *R. alboacuminatus* were a nuisance for the affected orchardist, who was worried about the negative impact on his business. The spread of *R. alboacuminatus* via farm equipment, vehicles, and transportation of farm produce is likely; we had to remove *R. alboacuminatus* hitchhikers from our vehicle before leaving the infested property. Vouchers of the three species have been deposited at the Canadian National Insect Collection, and Spencer and Royal BC museums. A *R. vulgaris* voucher from the invasive alien species landfill traps has been deposited in the Canadian Forest Service, Pacific Forestry Centre reference collection, Victoria, British Columbia. Photographs are of specimens collected at the sites.

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NATURAL HISTORY AND OBSERVATIONS

Notes on insects recently introduced to Metro Vancouver and other newly recorded species from British Columbia**C. G. RATZLAFF¹, K.M. NEEDHAM, and G. G. E. SCUDDER****ABSTRACT**

Sixteen insects species are recorded for the first time from British Columbia, including seven new to Canada. These records are comprised of nine introduced species, including the first record of *Rhopalum gracile* Wesmael (Hymenoptera: Crabronidae) from North America, and range extensions for seven species native to the Nearctic region. Colour images of selected specimens are included.

Key words: Introduced, Coleoptera, Diptera, Hemiptera, Hymenoptera, Trichoptera, British Columbia, Canada

INTRODUCTION

During the course of our fieldwork over the past few years, and by determining older unidentified material in the Spencer Entomological Collection, we have discovered 16 species never before recorded from British Columbia, including seven species new to Canada. Nine of the species have been introduced from their native ranges, and seven are range extensions for Nearctic species. We report these new records here.

Our field work during this time consisted mainly of monthly forays to local areas, participation in bioblitzes and species surveys outside the Lower Mainland (which concentrate efforts on a select locale for a short period of time), and an ongoing survey of the "green roof" atop the Vancouver Convention Centre.

All specimens recorded here are deposited in either the Spencer Entomological Collection [SEM] or the first author's personal collection [CGR]. All photos were taken by the first author using a Leica DFC490 digital camera mounted on a Leica M205C stereomicroscope. Post-processing of the images was done using Adobe Photoshop CS4.

INTRODUCED INSECTS

The following nine species are native to the Palearctic region and have been recently introduced to British Columbia. Three of these are new to Canada.

Coleoptera: Chrysomelidae***Cassida rubiginosa* Müller**

The leaf beetle, *Cassida rubiginosa* Müller, has been recorded from British Columbia for the first time. Native to Europe and Asia, this species was first introduced to North America in Quebec around 1902 and is now found throughout most of Canada east of the Rockies and in the northeastern United States. Members of the Family Asteraceae, specifically thistles, are the preferred host plant for *C. rubiginosa*, although they are polyphagous (Majka and Lesage 2008). The specimens collected in Richmond at both Terra Nova Rural Park and Iona Beach Regional Park were found feeding on thistle.

New Records: 3 adults, Richmond, Terra Nova Rural Pk., 49.1660, -123.1956, 15.viii.2013 (C. G. Ratzlaff) [CGR, SEM]; 1 adult, Richmond, Iona Beach Reg. Pk., 49.21940, -123.20839, 12.ix.2014 (C. G. Ratzlaff) [CGR]; 1 adult, Richmond, Iona

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Beach Reg. Pk., 49.2196, –123.2083, 13.v.2015 (C. G. Ratzlaff) [CGR] (Fig. 1a); 1 adult, Richmond, Iona Beach Reg. Pk., 49.220, –123.211, 1.vii.2015 (C. G. Ratzlaff) [SEM]

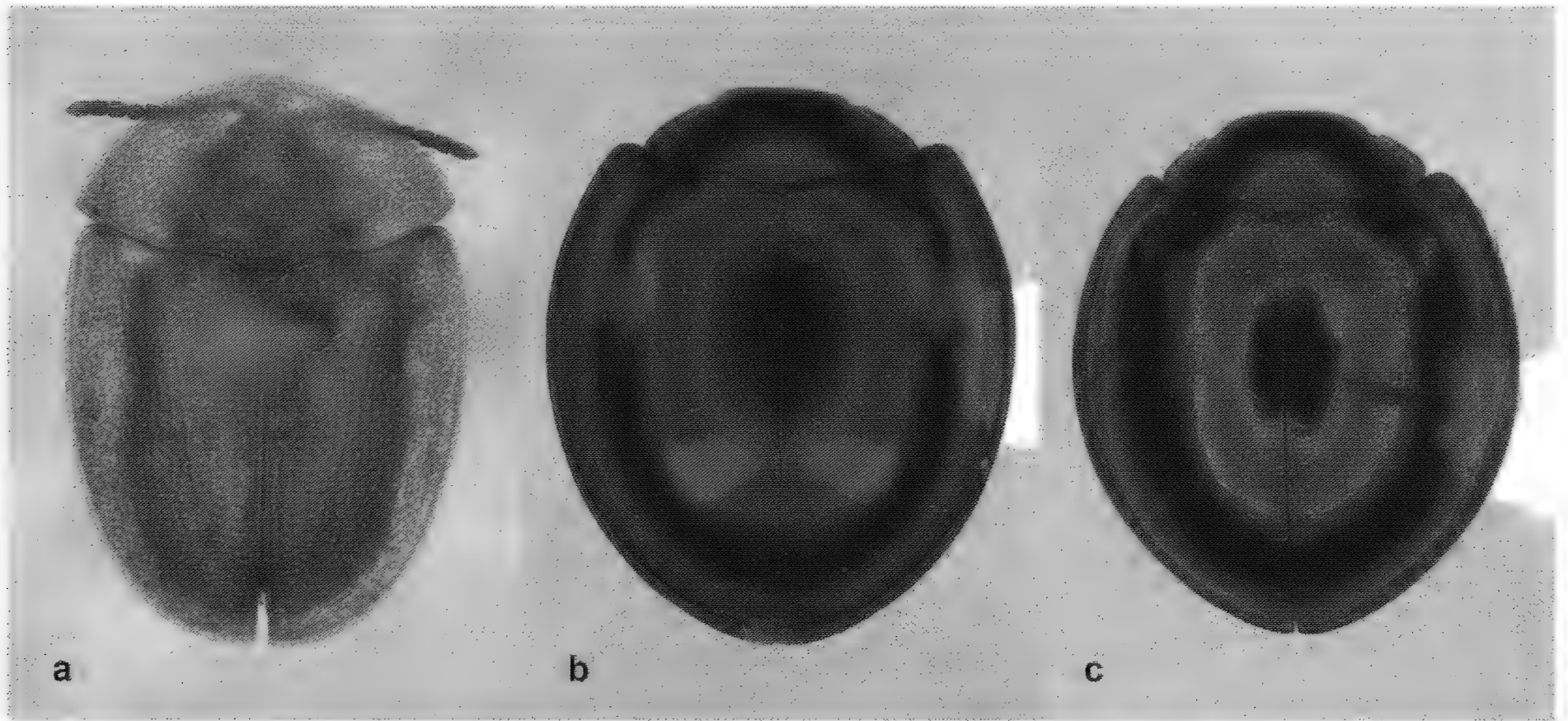


Figure 1. (a) *Cassida rubiginosa* Müller from Iona Beach Regional Park, in Richmond, B.C.; (b) *Brumus quadripustulatus* Linnaeus from the University of British Columbia (UBC) Botanical Garden, in Vancouver, B.C.; and, (c) *Chilocoris bipustulatus* (Linnaeus) from the Beaty Biodiversity Museum at UBC, in Vancouver.

Coleoptera: Coccinellidae

***Brumus quadripustulatus* Linnaeus**

The pine ladybird, *Brumus quadripustulatus* Linnaeus, used widely in the Palearctic region as a biocontrol agent, was first introduced and established in California between 1915 and 1928 as a control for woolly hemlock adelgid (Gordon 1985). There are no known further releases of *B. quadripustulatus*, and the species has apparently dispersed north along the Pacific Coast. Year-round specimen records show that this species can overwinter as an adult. These represent the first records of this species in Canada.

New Records: 1 adult, Richmond, 49.1888, –123.0959, 16.vi.2013 (C. G. Ratzlaff) [CGR]; 1 adult, Vancouver, 49.2281, –123.0870, 13.iv.2014 (C. G. Ratzlaff) [CGR]; 2 adults, Vancouver, UBC Botanical Garden, 49.254, –123.247, 16.v.2014 (C. G. Ratzlaff) [CGR, SEM] (Fig. 1b); 1 adult, Vancouver, UBC Botanical Garden, 49.254, –123.247, 4.xi.2014 (C. G. Ratzlaff) [CGR]; 1 adult, Vancouver, 49.2281, –123.0870, xi.2014 (C. G. Ratzlaff) [CGR]; 1 adult, Vancouver, Pacific Spirit Reg. Pk., 49.2445, –123.2002, 8.ii.2016 (C. G. Ratzlaff) [SEM]; 1 adult, Vancouver, UBC, Beaty Biodiversity Museum, 49.2632, –123.2502, 22.iii.2016 (C. G. Ratzlaff) [SEM]; 2 adults, Vancouver, UBC, Beaty Biodiversity Museum, 49.2632, –123.2502, 1.iv.2016 (C. G. Ratzlaff) [SEM]; 2 adults, New Westminster, Sapperton Landing Pk., 49.2166, –12.8929, 11.iv.2016 (C. G. Ratzlaff) [SEM]; 1 adult, Burnaby, Deer Lake Pk., 18.viii.2016 (K. Needham) [SEM]

Coleoptera: Coccinellidae

***Chilocoris bipustulatus* (Linnaeus)**

Chilocoris bipustulatus (Linnaeus), a species widespread in the Palearctic region, first became established in North America when it was introduced in California in 1951 as a biocontrol agent for scale insects (Gordon 1985). Although it likely has been released in a number of locations throughout North America, *C. bipustulatus* is poorly adapted to low temperatures and will not survive cold winters (Kehat *et al.* 1970). Our records from Vancouver, the first for Canada, may be the direct result of biocontrol releases where recent mild winters may have enabled this species to persist. Our first specimens were

collected at Burns Bog during a B.C. Ministry of Environment, Lands, and Parks Ecosystem Review (Kenner and Needham 1999), but were misidentified as *Chilocoris tricyclus* Smith, a similar-looking native species.

New Records: 3 adults, Delta, Delta Nature Reserve, 11.x.1999 (R. Kenner and K. Needham) [SEM]; 1 adult, Vancouver, Queen Elizabeth Pk., 49.2442, -123.1121, 15.v.2013 (C. G. Ratzlaff) [SEM]; 1 adult, Vancouver, 49.2281, -123.0870, 17.x.2013 (C. G. Ratzlaff) [CGR]; 1 adult, Vancouver, UBC Botanical Garden, 49.254, -123.247, 17.iv.2015 (C. G. Ratzlaff) [SEM]; 2 adults, Vancouver, UBC, Beaty Biodiversity Museum, 49.2632, -123.2502, 19.iv.2016 (C. G. Ratzlaff) [SEM] (Fig. 1c)

Diptera: Opomyzidae

Geomyza tripunctata (Fallén)

The Palearctic opomyzid, *Geomyza tripunctata* (Fallén), commonly known as the cereal fly, is recorded for the first time in British Columbia. In Canada, it has previously been recorded from Ontario, Quebec, Prince Edward Island, and Nova Scotia (Wheeler *et al.* 1999). The larvae feed on the shoots of various grasses and have the potential to be a crop pest.

New Records: 1 adult, Vancouver, 49.2281, -123.0870, ix.2010 (C. G. Ratzlaff) [CGR]; 1 adult, Vancouver, 49.2281, -123.0870, 27.vi.2013 (C. G. Ratzlaff) [SEM]; 1 adult, Abbotsford, 49.0782, -122.3130, 18.viii.2013 (C. G. Ratzlaff) [SEM]; 1 adult, Vancouver, Memorial South Pk., pond, 49.2321, -123.0869, 5.ix.2013 (C. G. Ratzlaff) [CGR]; 1 adult, Richmond, Iona Beach Reg. Pk., 49.2276, -123.2299, 11.iv.2014 (C. G. Ratzlaff) [CGR]; 1 adult, Mayne I., Miner's Bay, 48.8504, -123.3013, 15.xi.2014 (C. G. Ratzlaff) [SEM]; 2 adults, Vancouver, Vancouver Convention Centre, Green Roof, 49.2887, -123.1162, 8.iv.2016 (C. G. Ratzlaff and K. Needham) [SEM] (Fig. 2a); 1 adult, Sidney I., Dragonfly Pond, 48.6033, -123.3046, 14.viii.2016 (SEM Team) [SEM]

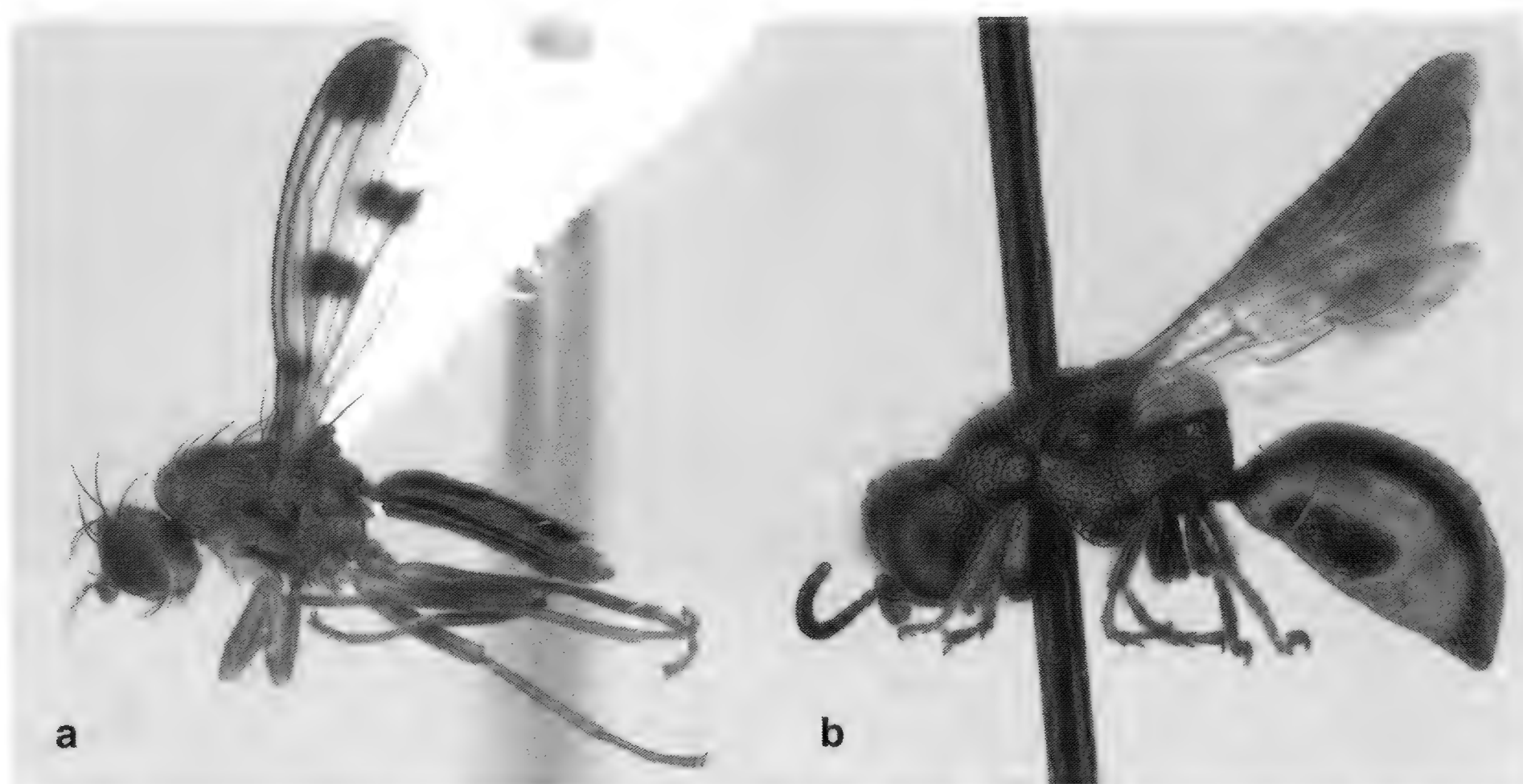


Figure 2. (a) *Geomyza tripunctata* (Fallén) from the green roof at the Vancouver Convention Centre, Vancouver, B.C.; (b) *Pseudomalus auratus* (Linnaeus) from Woods Island Park, in Richmond, B.C.

Hemiptera: Miridae

Atractotomus magnicornis (Fallén)

The plant bug, *Atractotomus magnicornis* (Fallén), is recorded for the first time in British Columbia. Native and widespread in Europe, *A. magnicornis* is currently found in Ontario, Quebec, Nova Scotia, and Newfoundland, as well as in several states in the

eastern United States (Stonedahl 1990; Maw *et al.* 2000). This species is associated with conifers, primarily members of *Abies* and *Picea*, often in gardens or other ornamental environments (Stonedahl 1990).

New Records: 1♀, Coquitlam, Westwood Plateau Ridge Pk., 6.vii.2013 (T. Loh) [SEM]; 3♀, Vancouver, UBC, Beaty Biodiversity Museum, 49.2632, -123.2502, 15.vii.2016 (C. G. Ratzlaff) [SEM]

Hymenoptera: Chrysididae

Chrysis angolensis Radoszkowski

The large cuckoo wasp, *Chrysis angolensis* Radoszkowski, a cleptoparasite of mud-daubers in the genus *Sceliphron*, is recorded for the first time in British Columbia. A common host, *Sceliphron caementarium* (Drury), often builds mud nests on human-made structures that are then transported and has been spread throughout many parts of the world, facilitating the cosmopolitan distribution of *C. angolensis* (Kimsey 2006). In North America, *C. angolensis* is thought to have been originally introduced to the eastern US during World War II and has since spread to the western states, eastern Canada, and parts of Mexico (Bohart and Kimsey 1982).

New Records: 1♀, Vancouver, UBC Campus, 49.2593, -123.2477, 12.ix.2013 (C. G. Ratzlaff) [SEM]; 1♀, Richmond, Terra Nova Rural Pk., 49.173, -123.199, 4.x.2015 (J. Chan) [SEM] (Fig. 3)



Figure 3. Female *Chrysis angolensis* Radoszkowski collected from Terra Nova Rural Park, in Richmond, B.C.

Hymenoptera: Chrysididae

Pseudomalus auratus (Linnaeus)

The small cuckoo wasp, *Pseudomalus auratus* (Linnaeus), has recently been introduced to the Vancouver area. Native to Europe, Asia, and North Africa, this species was introduced to the eastern United States sometime before 1828. It was recorded from Utah in the late 1960s and then in California in 1980 (Bohart and Kimsey 1982). It has since spread to a number of other states, as well as to eastern Canada. The hosts for *P. auratus*, a cleptoparasite, are stem-nesting pemphredonine (Crabronidae) wasps, and it has been suggested that its spread is a result of the transportation of garden plants (Danks 1971; Bohart and Kimsey 1982).

New Records: 1♂ 2♀, Vancouver, 49.2281, -123.0870, 17.vi.2013 (C. G. Ratzlaff) [CGR, SEM]; 1♂, Vancouver, 49.2281, -123.0870, 10.vi.2014 (C. G. Ratzlaff) [SEM]; 1 adult, Richmond, Iona Beach Reg. Pk., 49.22177, -123.21223, 25.vi.2014 (C. G. Ratzlaff) [CGR]; 1 adult, Vancouver, 49.2281, -123.0870, 29.vi.2014 (C. G. Ratzlaff) [CGR]; 1 adult, North Saanich, Schwartz Bay Terminal, 49.6883, -123.4099, 17.vii.2015 (C. G. Ratzlaff) [CGR]; 1 adult, Vancouver, 49.2281, -123.0870, 6.v.2016 (C. G. Ratzlaff) [SEM]; 1 adult, Richmond, Woods I. Pk., 49.2119, -123.1646, 14.vii.2016 (C. G. Ratzlaff) [SEM] (Fig. 2b)

Hymenoptera: Crabronidae

Rhopalum gracile Wesmael

The crabronid wasp, *Rhopalum gracile* Wesmael, is recorded here for the first time in North America from Iona Beach Regional Park in Richmond, British Columbia. This small wasp lives throughout the Palearctic region from England to Japan, although it is typically uncommon (Bitsch and Leclercq 2009). It nests in the stems of plants such as *Euthamia occidentalis* Nuttall and species of *Phragmites* (Bitsch & Leclercq 2009); the latter genus grows near the specimen collection location. *Rhopalum gracile* was probably introduced by means of aircraft, because Iona Beach is close to Vancouver International Airport. It is unknown whether a population will become established; further monitoring is needed.

New Record: 1♂ 1♀, Richmond, Iona Beach Reg. Pk., 49.2196, -123.2083, 22.vii.2015 (C. G. Ratzlaff) [SEM] (Fig. 4)



Figure 4. *Rhopalum gracile* Wesmael (a) male and (b) female from Iona Beach Regional Park, in Richmond, B.C.

Hymenoptera: Torymidae

Megastigmus aculeatus (Swederus)

The parasitic wasp, *Megastigmus aculeatus* (Swederus), is recorded for the first time in western North America. This species develops in the seed buds of *Rosa* species and reproduces primarily through thelytokous parthenogenesis. Although native to western Europe, *M. aculeatus* has spread over much of the globe and now occurs throughout eastern Europe, parts of Africa, Iran, Iraq, China, Japan, Australia, and eastern North America, including Ontario and Quebec. More than 23 host species of *Rosa* have been recorded in the West Palearctic region, including several that have been introduced to North America (Roques and Skrzypczyńska 2003). The wide commercial transport of *Rosa* species, along with their parthenogenic reproduction, allow for easy colonization of areas by *M. aculeatus*.

New Record: 1 ♀, Whistler, Creekside Field, 50.0942, -122.9874, 9.vii.2016, 650m, light trap (SEM Team) [SEM] (Fig. 5)



Figure 5. Female *Megastigmus aculeatus* (Swederus) from Creekside, in Whistler, B.C.

SIGNIFICANT RANGE EXTENSIONS OF NEARCTIC INSECTS

The following seven species are native to North America; these records represent range extensions into British Columbia. Four of these species are also new to Canada.

Diptera: Blephariceridae

***Philorus californicus* (Hogue)**

The net-winged midge, *Philorus californicus* (Hogue), is recorded for the first time in Canada. This also represents the first record of the genus in Canada. Members of this family of flies are associated with fast-flowing streams, where the larvae live on the surfaces of rocks, often in cracks and crevices (Hogue 1973).

New Record: 1 larva, Lindell Beach, nr. Stillwood Camp, Watt Cr., 49.024, -121.999, 17.vi.2014, under rock in fast-flowing stream (C. G. and N. A. Ratzlaff) [SEM] (Fig. 6)

Diptera: Ulidiidae

***Chaetopsis massyla* (Walker)**

The picture-wing fly, *Chaetopsis massyla* (Walker), is recorded for the first time from British Columbia, collected during the 2015 Gulf Islands National Park Reserve BioBlitz on Saturna Island. This species is widespread throughout most of North America and feeds on damaged stems of wetland monocots, such as cattails and sedges (Steyskal 1965; Allen and Foote 1992). *Chaetopsis massyla* is also a pest of corn in several south-eastern states (Goyal *et al.* 2010).

New Record: 1 adult, Saturna I., Gulf Islands Nat. Pk. Res., Winter Cove, 48.8123, -123.1879, 17.vii.2015 (C. G. Ratzlaff) [SEM] (Fig. 7)

Hemiptera: Miridae

***Orectoderus montanus* Knight**

The plant bug, *Orectoderus montanus* Knight, is recorded for the first time in British Columbia. The genus *Orectoderus* Uhler was recently revised and now contains five

species, with three of those species reported from Canada (Nyniger 2010). To date, *O. montanus* has been recorded in Canada only from Alberta and Saskatchewan (Kelton 1980; Maw *et al.* 2000; Nyniger 2010). In the United States, it has been recorded from Colorado, Idaho, Montana, Nevada, North Dakota, Utah, and Wyoming. Hosts for *O. montanus* include *Ericameria nauseosa* (Pall. ex Pursh) (Asteraceae), *Symphoricarpos* spp. (Caprifoliaceae), and *Potentilla fruticosa* Linnaeus (Roseaceae) (Nyniger 2010).

New Record: 1♂, Pink Mt., 57.0487, -122.8687, 2.vii.2016, 1715m (C. G. and N. A. Ratzlaff) [SEM]

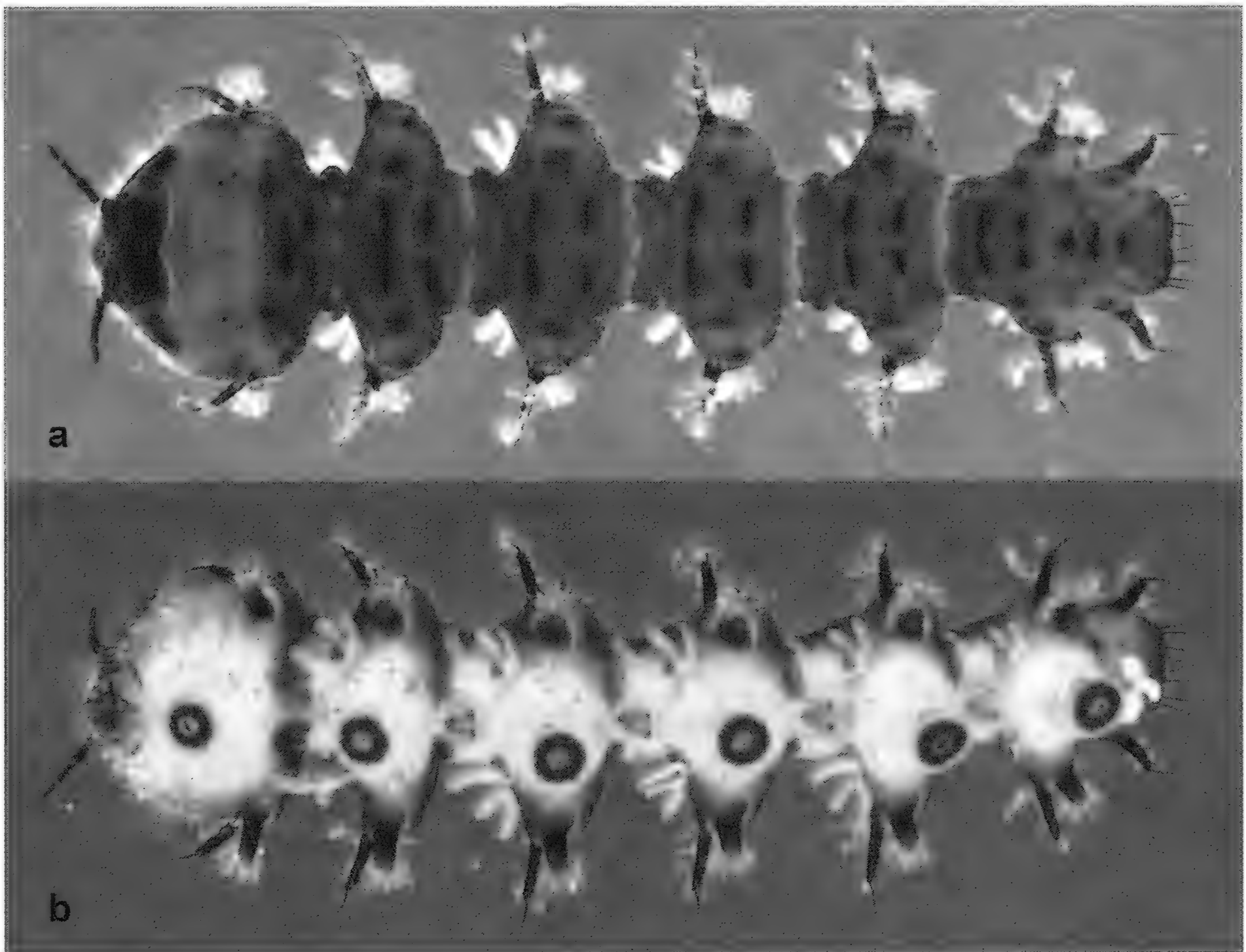


Figure 6. (a) Dorsal and (b) ventral view of a *Philorus californicus* (Hogue) larva from Watt Creek, south of Cultus Lake, B.C., showing the suction cups on each segment used to hold onto the surface of rocks in fast-flowing streams.

Hymenoptera: Crabronidae

Spilomena barberi Krombein

The small cryptic wasp, *Spilomena barberi* Krombein, is identified for the first time from British Columbia. It has been recorded in Canada, from Ontario and Quebec, and in the United States, from coast to coast (Buck 2004). Little is known about the biology of *S. barberi*, and it is rarely collected.

New Record: 1♀, Osoyoos, Haynes Ecological Reserve, 13.vii. - 17.viii.1988, pitfall trap, *Purshia/Aristida* shrub-steppe (S.G. Cannings) [SEM]; 1♀, Hornby Island, Norman Pt., 9.vii.1989 (S. G. Cannings) [SEM]

Hymenoptera: Platygasteridae

Synopeas anomaliventris (Ashmead)

The parasitic wasp, *Synopeas anomaliventris* (Ashmead), is identified for the first time from Canada. It has previously been recorded from Maryland, Pennsylvania, Florida, Louisiana, and New Hampshire, in the eastern United States (Muesebeck 1979).

Many small parasitic wasps have widespread distributions, and whether this disjunct record is natural or the result of an accidental introduction is unknown. Nothing is known of the biology of this species, but others in the genus are parasitic on flies in the Family Cecidomyiidae (Fouts 1924).

New Record: 1♀, Galiano I., north end, 19.iv.1981 (S. G. Cannings) [SEM]



Figure 7. *Chaetopsis massyla* (Walker) from Winter Cove in Gulf Islands National Park Reserve, on Saturna Island, B.C.

Hymenoptera: Pompilidae

Ceropales pacifica Townes

The spider wasp, *Ceropales pacifica* Townes, is recorded from Canada for the first time, previously being recorded only from Oregon and California. No specific information about the biology of *C. pacifica* is known, but members of the genus are kleptoparasites of other wasps in the Family Pompilidae. Females lay an egg in the book lungs of a host wasp's unattended spider prey before it is deposited in the host's nest. When hatched, the *Ceropales* larva will consume both the spider and the egg laid by the host wasp (Townes 1957).

New Records: 1♂1♀, Oliver, UBC Geology Camp, 20.vii.1989, malaise trap, pine/thicket edge (S. G. Cannings) [SEM]; 2♂, Oliver, UBC Geology Camp, 22.vii.1989, malaise trap, pine/thicket edge (S. G. Cannings) [SEM]; 1♂, Oliver, UBC Geology Camp, 22.vii.1990, malaise trap, ponderosa pine forest (S. G. Cannings) [SEM]; 1♀, Oliver, UBC Geology Camp, 27.vii.1990, malaise trap, hawthorn thicket edge (S. G. Cannings) [SEM] (Fig. 8); 1♂, Oliver, UBC Geology Camp, 28.vii.1990, malaise trap, hawthorn thicket edge (S. G. Cannings) [SEM]

Trichoptera: Limnephilidae

Desmona mono (Denning)

Desmona is an unusual genus of caddisfly in the Family Limnephilidae. Its members inhabit the shallow edges of high-alpine lakes and springs or seeps of alpine meadows (Wiggins 1996). Although most limnephilids are detritivores, feeding on dead and decaying plant material at the bottom of these lakes, *Desmona* crawl out of the water at night to feed on living plants near the water's edge (Wiggins and Wisseman 1990).

In North America, three species are recognized in the genus (Nimmo 2012). *Desmona bethula* Denning and *Desmona denningi* Nimmo are found in California, while *Desmona*

mono (Denning) (= *Monophylax mono*) inhabits the Pacific Northwest of the United States.

During surveys of some high-alpine lakes, streams, and seeps atop Whistler Mountain as part of the 2014 Whistler Bioblitz, we collected several larvae and cases of *Desmona*, which were later identified as *D. mono*. These represent the first record of this genus and species for British Columbia and Canada.

New Records: 9 larvae, Whistler, Blackcomb Mt., Overlord Trail wetland, 50.080, -122.888, 23.viii.2014 (C. M. Stinson) [SEM] (Fig. 9); 3 larvae, Whistler, Whistler Mt., Harmony L., 50.0640, -122.9379, 3.ix.2014, 1760m (C. G. Ratzlaff) [SEM]; 8 larvae, Whistler, Whistler Mt., Harmony L., 50.0640, -122.9379, 28.ix.2014, 1780m (C. G. Ratzlaff) [SEM]; 1 larva, Whistler, Whistler Mt., alpine stream, 50.0522, -122.9350, 28.ix.2014, 1810m (C. G. Ratzlaff) [SEM]; 1 larva, Whistler, Blackcomb Mt., Blackcomb L., 50.0813, -122.8812, 27.vi.2015, 1800m (C. G. Ratzlaff) [SEM]



Figure 8. *Ceropales pacifica* (Townes) from the UBC Geology Camp, in Oliver, B.C.

CONCLUSION

Examination of these new records highlights the importance of concentrated surveys targeting specific locales, as well as the usefulness of repeated forays to the same area. Seasonal and yearly variation in species presence makes it imperative to revisit a site periodically in order to produce a comprehensive species list for an area.

The value of the taxonomic expertise of previous researchers and detailed reference material, such as exists in provincial and national research collections, cannot be underestimated in projects such as these. Cataloguing the biodiversity of an area would not be possible without these resources. Adding to the historical record of species distributions and seasonal occurrences is valuable both in and of itself and as a foundation for further research.

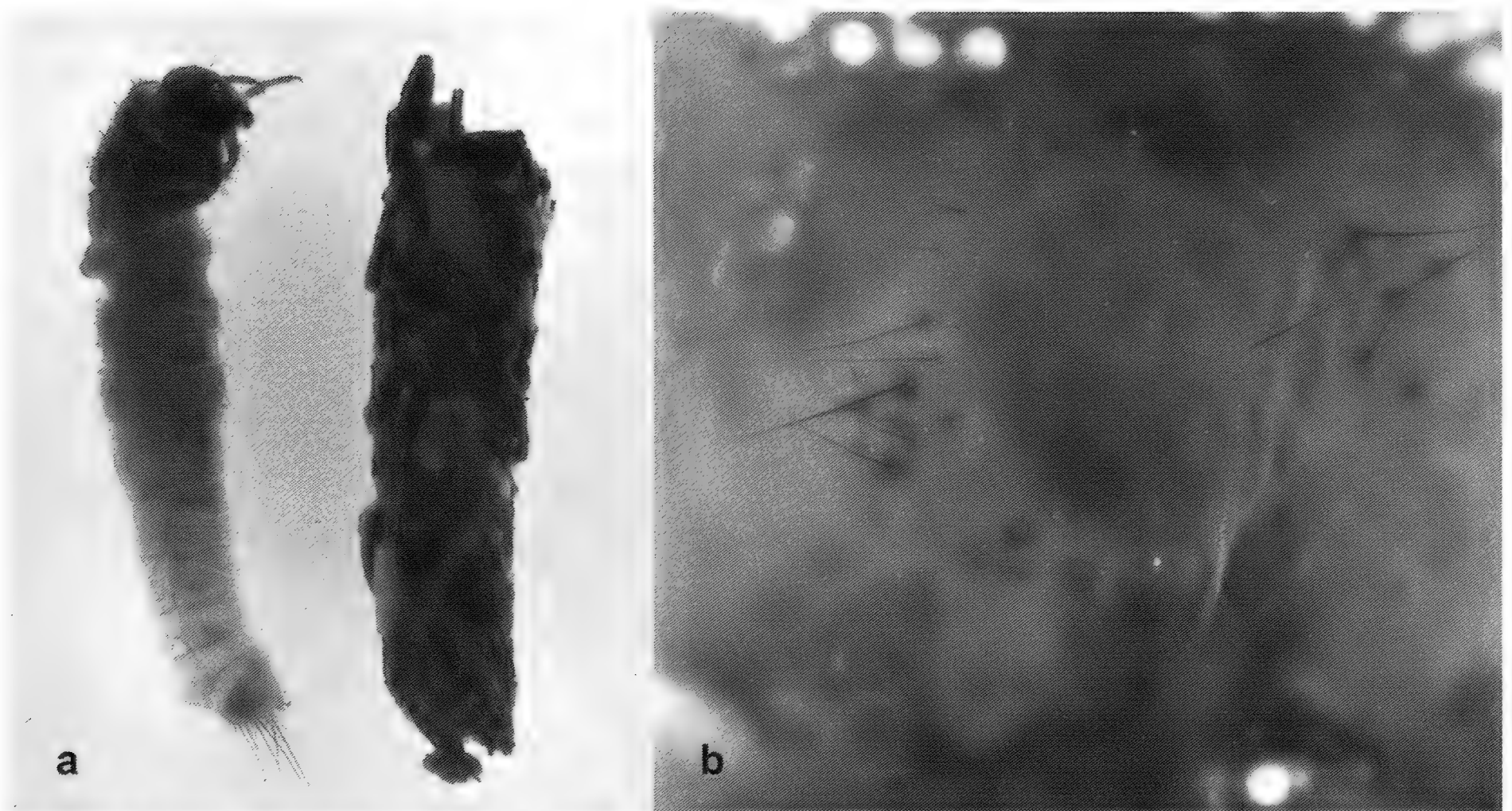


Figure 9. (a) Larva and case of *Desmona mono* (Denning) from Overlord Trail on Blackcomb Mountain, in Whistler, B.C.; (b) The lateral hump showing the two ring-like sclerites found in the larvae of *Desmona* species.

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NATURAL HISTORY AND OBSERVATIONS

***Rhyparochromus vulgaris* (Schilling) (Hemiptera: Heteroptera: Rhyparochromidae): newly discovered in the interior of British Columbia**

G. G. E. SCUDDER¹

The Palaearctic seed bug *Rhyparochromus vulgaris* (Schilling) was first reported from Oregon and Washington in North America by Henry (2004). The species is here reported for the first time in the interior of British Columbia (B.C.).

In early 2015, Mike Art observed many specimens in a woodshed in Creston, B.C. Through the cooperation of Ward Strong, Arthur Stock and Karen Needham, Kristine Saceniaks collected a series of specimens for me on 1 May 2015, at Creston, Goat River, 851 Aldich Road 49.075986°N 116.517452°W (UTM 11 –0535241E x 5436015W) at an elevation of 546 metres. Using the keys and description in Péricart (1998) and comparison with specimens from several localities in Europe, I confirmed that the species was *Rhyparochromus vulgaris* (Schilling) (Fig. 1). The sample received contained nineteen males and four females of *Rhyparochromus vulgaris*, plus two males of *Arhyssus scutatus* (Stål) (Rhopalidae). This is the first record of *Rhyparochromus vulgaris* from the interior of British Columbia. However, previous to this, Dr. M. D. Schwartz (Ottawa) had identified three males and six females of *Rhyparochromus vulgaris* for the Canadian Food Inspection Agency (CFIA) that had been collected at a commercial nursery near Langley, B.C., on 28 February 2013. These were discovered in an outdoor covered storage area near boxes of supplies. These specimens are deposited in the Canadian National Collection of Insects (CNC). The Creston specimens have been deposited in the CNC, Royal British Columbia Museum (RBCM), University of British Columbia (UBC), and my own collection.

According to Péricart (1998), *Rhyparochromus vulgaris* occurs naturally throughout Europe and the Mediterranean region, from Belgium, Germany and Russia in the north around the 55th parallel, to Asia Minor in the south. Péricart (1998) had no records for the British Isles.



Figure 1. *Rhyparochromus vulgaris* (Schilling). B.C. specimen, male, dorsal view. Length 6.75 mm.

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Proceedings of the Pollination Science and Stewardship Symposium

Okanagan College Campus, Penticton, British Columbia, March 17, 2016

INTRODUCTION

Jennifer Heron, Cory S. Sheffield, and Cara Dawson

Pollination is a vital ecological process in terrestrial ecosystems. Pollinators are the organisms that provide this service, thus facilitating reproductive success in plant communities. Awareness of the importance of pollinators and pollination has increased in the last few decades, largely due to global declines in honey bee colonies and documented widespread declines of some bumble bee and other pollinator species. In Canada, the main pollinators are insects, with thousands of species from a wide range of taxa that regularly visit flowers. Among the most familiar and important insect pollinators are the butterflies and moths (Lepidoptera), many groups of flies (Diptera) and beetles (Coleoptera), and the Hymenoptera, which include the bees and many other types of wasps.

The symposium, Pollination Science and Stewardship, featured 12 presenters who spoke about current research and on topics relating to the diversity, conservation, ecosystem services, pesticide management, agriculture, citizen science, and stewardship for pollinators, with focus on Canada. The workshop also aimed to facilitate connections between pollination specialists and land managers, owners, stewards and biologists, thus enabling information and idea exchange such that these practitioners could then apply it to their own conservation work. More than 90 people attended the symposium. Participants included members of academia, industry professionals, agriculturalists, citizen scientists, artists, students, gardeners, and landowners interested in enhancing their properties for pollinators.

The symposium was supported by funding from Environment and Climate Change Canada Habitat Stewardship Program for Species at Risk, the British Columbia Ministry of Environment, the Royal Saskatchewan Museum, and the Entomological Society of British Columbia.

Butterflies, conservation, and citizen science in Canada

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Butterflies are colourful, relatively easy to identify, and popular with naturalists. Butterflies are also potential pollinators, even though they appear to be relatively delicate and clean when compared to furry, flower-wrestling, pollen-covered bees and syrphid flies. Recent work, however, shows the importance of non-syrphid flies to pollination (Orford et al. 2015, DOI: 10.1098/rspb.2014.2934), and by analogy we know little about the importance of butterflies. What we care about as biodiversity conservationists, however, is not just pollinators, or the “ecosystem service” of pollination, but the diversity and abundance of wild flowers and flower-visiting insects. In this regard, butterflies are ideal “flagship” organisms for the conservation cause.

Butterfly collecting laid the groundwork for the understanding of butterfly faunistics, and amateur collecting can be considered the original butterfly citizen science project. The non-consumptive approach to butterfly citizen science began in Canada as “Fourth of July Butterfly Counts” some 20 years ago, coordinated originally by the Xerces Society, and more recently by the North American Butterfly Association. There are still a

few such counts, including the Dry Island Buffalo Jump Provincial Park Butterfly Count, in Alberta. This count involves park rangers with nets, and counters the common perception that nets are evil. However, the data from such counts is poor, because of the effect of weather on shifting phenologies and the difficulty of comparing a one-day sample from one year to similar samples from other years. A better approach is to conduct weekly Pollard Walks, along a standard transect or route. This method results in much more valuable data, but it requires considerable effort, and has not caught on in Canada, with a few exceptions, including my own Pollard route in Edmonton, which I have visited regularly during the butterfly seasons since 1999.

Another approach involves the creation of butterfly atlas projects, such as the British Columbia Butterfly Atlas, the Ontario Butterfly Atlas, and the Maritimes Butterfly Atlas. These projects update both distributional and phenological databases, and they can be popular. In Alberta, the Alberta Lepidopterists' Guild (ALG) considered an atlas project, but declined because of 1) a small number of potential participants for such a large geographic area, 2) the fact that atlas projects are not open-ended, and are intended to produce a book-like end product, and 3) the perceived redundancy of atlassing and the citizen science project eButterfly.org.

Instead of an atlas, the ALG initiated the Alberta Butterfly Roundup in the spring of 2015, an open-ended attempt to reconfirm the 173 species of butterflies known from the province. In the 2015 season, 53 naturalists participated, and of those, 33 contributed at least one species to the count. A total of 120 species were reported, and the top contributor found 24 of these. The Roundup helped document a new species for the province (regal fritillary, *Speyeria idalia*) and the westward spread of a "native" species, the dun skipper (*Euphyes vestris*). Although participants were encouraged to submit records through eButterfly, most records were submitted through the ALG Facebook Page and the ALG listserver (Albertaleps), both two to three times as popular as eButterfly. eButterfly received about the same number of submissions as the Albertabugs listserver, the Edmonton Natural History Club listserver, and emails directly to me. In the future, and to find the remaining 52 species, ALG is encouraging directed searches for species that are rare and localized in areas that are not often visited. The Roundup approach may not possess the same scientific rigour as an atlas project, but it does focus attention on butterflies, with potential benefits for conservation, and the monitoring of pollinators and floral resources in general.

Biology and diversity of moths in Canada: a conservation perspective

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Butterflies and moths comprise the order Lepidoptera, one of the four most diverse orders of insects. Moths make up about 90% of Lepidoptera; the butterflies are just one small branch of the group. Approximately 5,100 species of moths live in Canada, with about 2,650 species known in B.C. Most moth larvae feed on living plant tissue, as exposed or concealed feeders on leaves, or as borers in stems, roots, flowers, and fruit.

Not all moths feed as adults, but those that do are looking for food energy such as nectar from flowers, which makes them potential pollinators. Many diurnal species are generalist flower visitors, but some species are very specialized. Males of many diurnal micromoth species, such as choreutids (Choreutidae) and fairy moths (Adelidae), patrol around nectar sources to find females, and exploit whatever flower species are present. Other moths, such as *Greya* and *Lampronia* (Prodoxidae), are more specialized. Females lay eggs in the flowers of their host plants, and they have been observed pollinating while ovipositing. In yucca moths (the Genus *Tegeticula* in the Prodoxidae), this specialization has developed into the famous mutualism between moths and plants. Yucca plants are completely dependent on yucca moths for pollination. Female yucca moths deposit pollen on the flower stigma after injecting eggs into the yucca flower ovary. The larvae then hatch and feed in the developing seed pods of the yucca plant. Thus, they require

pollination of the flower for their food to develop. The larvae eat only some of the developing seeds, leaving some to produce the next generation of plants. This text-book case of mutualism gets more complicated; two species of "cheating" yucca moths have evolved. These cheaters lay eggs on developing seeds that have been fertilized by the pollinator moth species, so they are completely dependent on the yuccas and the pollinating yucca moth species.

Many orchids rely on specialized moth pollinators that have co-evolved with the plants. Some orchids have pollinia—sticky anther tips that break off and attach to the visiting insect—to aid pollen transfer. Although moth–orchid pollination information is often scanty, several moth species have been observed with pollinia attached, so they clearly play a role in orchid pollination. Some orchids have evolved a system of placing the nectar at the base of a long spur, so the pollinating insect must reach deep into the plant and increase its chances of bumping the pollinia. This has triggered an evolutionary arms race where the insects evolve longer mouthparts to access nectar, and the plants evolve longer spurs to promote pollination. An extreme case is Darwin's Orchid (*Angraecum sesquipedale*), with a 40+ cm spur. At the time, no insect was known with long enough mouthparts to reach the nectar, and Charles Darwin predicted in 1862 that a moth must exist with the necessary mouthparts. It was discovered in 1902: *Xanthopan morgani praedicta* has a 40-cm-long proboscis.

Biology, conservation & stewardship for flies in Canada

Andrew Young, Carleton University, Ottawa, ON; email: a.d.young@gmail.com

Many species of true flies (Diptera), especially flower flies (Syrphidae), are significant wild pollinators. Diptera contribute approximately half of the pollination services in most environments and become increasingly important with increasing latitude. For example, flies are the primary (and often only) pollinators north of the Arctic Circle, but despite this their ecological impact has been historically underappreciated, and relatively little is known about the specifics of many plant–pollinator interactions. Potential conservation efforts are currently hampered by inadequate taxonomic knowledge, coupled with poor knowledge of distribution for most species. Even within the taxonomically well-known family Syrphidae, there is a need for increased collecting effort and development of widely accessible, user-friendly identification tools. Several case studies of potentially endangered species and conservation efforts are discussed.

Biology, conservation and stewardship for bees in Canada

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Bees are the most important pollinators of both crop and native plant species. Although a few managed species are thought to provide most of the pollination services to crops, a growing body of evidence suggests that wild bee species play an important role. This is especially true for native plant species, some of which have intimate relationships with their bee pollinators. Over 800 species of bee are recorded from Canada, most of these occurring in ecozones in the southern half of the country. Our recent efforts have focused on documenting the patterns of diversity and distribution of Canada's bees, this being facilitated with DNA barcoding. These initiatives have also allowed for the first comprehensive assessment of the conservation status of the bee fauna of Canada. However, there is still much to learn. This session covers our current knowledge of bees in Canada, including their diversity, distribution, gaps in taxonomic knowledge, and conservation status.

Global bee diversity and conservation

Laurence Packer, York University, Lumbers Building 345, 4700 Keele Street, Toronto, ON M3J 1P3, Canada; email: xeromelissa@gmail.com

More than 20,000 species of wild bee exist in the world, and almost none of them fit the standard archetype people have as to what bees are. Those archetypes relate to the domesticated honey bee, which is not native to North America; further, honey bees have been shown to often not be as important for pollination of crops as wild bees are. This presentation focuses on the taxonomic, ecological and behavioural diversity of the bees of the world, and compare bee diversity globally with that from Canada.

The Federal General Status Program of Canada, Wild Species 2015

Leah Ramsay, British Columbia Ministry of Environment, British Columbia Conservation Data Centre, Mezzanine Floor - 395 Waterfront Crescent, Victoria, B.C., Canada V8W 9M2; email: Leah.Ramsay@gov.bc.ca

The General Status Program arose from the Accord for the Protection of Species at Risk (1996) and one of the resulting sections of the *Species at Risk Act* (SARA), which include the requirement to assess and report regularly on the status of all wild species in Canada. The resulting amassing of data provides the baseline for assessing the status of all species in Canada—including invertebrates. A report is completed every five years and the results are published. The first step in the assessment process is the refinement of lists for Canada and each province and territory for the species group that is being reported on. The lists are based on published literature, museum collections and personal knowledge of experts. The next step involves pulling together whatever basic information is available on the distribution, trends, habitat, threats and range extent using the same sources as for the lists. These are some of the criteria that are used to determine a conservation status rank or general status rank.

The conservation status assessments for the 2015 Wild Species report were done following NatureServe methodology. This is the same process that is used for the status ranks that are provided by the Conservation Data Centres and Heritage programs within each of the jurisdictions. The factors, methods and the calculator are all found at www.natureserve.org. In British Columbia the results, including the resulting lists are held and maintained within the B.C. Conservation Data Centre. The first report in 2000 assessed 1,670 species (mainly vertebrates) and the 2015 report will assess approximately 30,000 (final number to be determined), including capsular and non-vascular plants and many groups of invertebrates. One of the focusses for the 2015 report was assessment of as many of the pollinator groups as possible, including the bumble bees, which. The bumble bees had been done initially in 2010, as well as the rest of the bees were assessed as well as moths, bee flies, beetles and wasps, to name a few. This presentation describes the assessment process and methodology, as well as discusses a number of ways the General Status Program has been used to improve knowledge and benefit species conservation in Canada.

The General Status program originated in order to fulfill the requirements of SARA of enabling a metric to use to determine overall trends in the biodiversity of species in Canada. Other beneficial offshoots include (but are not limited to), helping to inform assessment priorities for the Committee on the Status of Wildlife in Canada (COSEWIC), to establish a baseline of data for all species, identify knowledge gaps by highlighting species where little is known and further inventory is required and provide taxonomic lists for all of the jurisdictions and Canada. One can also get a snapshot of the diversity of different groups in the different regions of Canada as well as see where across the jurisdiction something may be declining or in good shape.

Pollination, pollinator diversity, and the determinants of plant reproduction

Jana Vamosi, Department of Biological Sciences, University of Calgary, 2500 University Drive NW, Calgary, AB T2N1N4; email: vamosi@ucalgary.ca

Understanding the role of pollinators in determining plant reproduction is critical for conserving and restoring ecosystems, as well as for maintaining food security. While some crop plants are self-compatible and require little input from pollinators to produce fruit and seeds, many plant species in natural systems require pollinators to effect pollen transfer. Recent research reveals that when many plant species are in an area, they receive inadequate pollination and produce suboptimal levels of fruit and seeds, yet the mechanisms behind these observed patterns are unclear (Vamosi *et al.* 2006). Species in species-rich areas may be more specialized, having traits that attract certain pollinators and restrict access of others (Vamosi *et al.* 2014). While these traits may confer advantages when a favoured pollinator is present, they also put the plant species at risk of lower reproduction if their specialist group of pollinators is absent. Here, I summarize a number of approaches how specialization may affect pollen delivery and conserve the ecosystem function of pollination.

The initial approach to evaluating whether a plant species is receiving adequate pollination service from pollinators is to estimate “pollen limitation”, typically through a manipulative experimental design where the proportion of fruits or seeds set under natural pollination conditions is compared to that under supplemental pollination conditions (Knight *et al.* 2005). Thousands of pollen limitation experiments have now been conducted throughout the world, with estimates that ~60% of species are pollen-limited. In other words, these pollen-limited species would produce more fruits or seeds if pollinators were optimally abundant. Reasons for this widespread pollen limitation are currently unclear but some have posited that the pattern is a reflection of an impending “pollination crisis” (Ingram *et al.* 2002), evidenced by the observations that pollinators are declining in abundance and diversity at an alarming rate (Potts *et al.* 2010). While pollen limitation can be observed to increase with disturbance and loss of pollinators (Da Silva *et al.* 2013), it is important to recognize that natural processes may also cause plant species to exhibit pollen limitation as well. For example, observational studies comparing the pollen limitation of species that were visited by diverse arrays of pollinators were often no less pollen limited than those populations that received visits from few species of pollinators (Davila *et al.* 2012), indicating that we do not fully understand the functional role of pollinator diversity in communities. In examining the contribution that differences in floral visitor composition make to increased selfing and seed production of plant populations, we find that increased visitation by particular functional groups ensures reproductive success of focal plant species more so than pollinator diversity (Adderley and Vamosi 2015). Thus, while entire flowering communities may benefit from functionally diverse pollinator communities, the reproductive success of a single pollinator species is more contingent on a specialized subset of pollinators.

Conversely, the prevalence of certain plant traits within a community can influence pollinator composition (Bruckman and Campbell 2014). In an investigation of the effects of changes in plant composition that altered the prevalence of zygomorphy (i.e., floral symmetry and the restrictiveness of flowers to certain pollinators), the conversion to grazing pasturelands negatively impacted species richness and phylogenetic diversity. Changes in community composition and structure had strong effects on the prevalence of zygomorphic species, likely driven by nitrogen-fixing abilities of certain clades with zygomorphic flowers (e.g., Fabaceae). Land conversion can thus have unexpected impacts on trait distributions relevant for the functioning of the community in other capacities (e.g., cascading effects to other trophic levels (i.e., pollinators) (Villalobos and Vamosi 2016). These patterns indicate that we may be able to predict which pollinators will be available to various crop plants by understanding their relationships with the floral community and climate (Kerr *et al.* 2015).

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Pollination in Agriculture: Insects and Ecological Intensification

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Agricultural expansion and intensification are central to the current demise of biodiversity, affecting plants, animals (including insects) and fungi. The new buzz-phrase “ecological intensification” goes beyond the restoration of biodiversity to the reestablishment of ecosystem functionality. According to Food and Agriculture Organization of the United Nations (FAO 2013) it is “a knowledge-intensive process that requires optimal management of nature’s ecological functions and biodiversity to improve agricultural system performance, efficiency, and farmers’ livelihoods”. Animal pollination accounts for approximately one-third of our food supply, and new data from around the world indicate that wild pollinators are far more important than managed ones in many cropping systems. Yield drags of 20–30% have been documented for numerous crops often stated to not require insect pollination, new cultivars are not tested for their pollination requirements and their floral ecology is generally ignored, and scant attention and little regard has been paid beyond bees to other insect groups essential to pollination ecosystem services. Landscape management in agricultural and urban environments that is focused on pollinator habitats has, at the same time, encouraged populations of biocontrol agents, notably parasitoids and some predators that depend on floral resources for part of their life cycles. The FAO’s call to “optimize management [as] a knowledge-intensive process that requires optimal management of nature’s ecological functions and biodiversity to improve agricultural system performance, efficiency and farmers’ livelihoods” strongly suggests intensive and well-informed human intervention. An example may be the use of managed pollinators to disseminate microbial biological control agents against crop pathogens and insects pests on crops. The multiple benefits of better yields through pollination plus crop protection that are coupled to reduced uses of chemical pesticides, conservation of water and less consumption of fossil fuels. The

knowledge deficits, changing perspectives, and current emerging practices around ecological intensification through biological control of pest diseases, IPM and pollination are discussed.

Agriculture in the Okanagan: past, present and future

Kenna MacKenzie, Agriculture and Agri-Food Canada, Summerland, B.C., 4200 Highway #97 South, Summerland, B.C. V0H 1Z0; email: Kenna.MacKenzie@agr.gc.ca

The Okanagan is the second most important agricultural region in British Columbia. In the late-1800s and early 1900s, agriculture began in the Okanagan with mixed farming, in particular beef cattle and tree fruits. Over the years, various crops and animals have been produced, such as vegetables, fruit, forage, beef and dairy, with tree fruits becoming predominant. Changing climate, with higher winter temperatures and longer growing seasons, has allowed a switch in crops. Today, tree fruits, particularly sweet cherry and apples, and wine grapes, are the main agricultural crops in valley. These trends are expected to continue in the near future.

Honeybee genetics and breeding a better honeybee

Brock Harpur and Amro Zayed, York University, Toronto, ON; email: Brock Harpur harpur@yorku.ca; Amro Zayed zayed@yorku.ca

The genome contains the evolutionary history of a given species. The modestly sized honey bee genome was sequenced in 2006, and since then many discoveries have been made about the honey bee's ancient history, its population expansions and adaptations, and its genetic health. Here, we present the findings of several major studies from our group that demonstrate a means through which both beekeepers and researchers can gain valuable information about the genetic health and history of the honey bees with which they work. First, we examine the evolution and genetic underpinnings of the honey bee immune system and uncover valuable insights into how novel forms of social immunity have evolved. Second, we demonstrate how, by applying genomic data such as this within the beekeeping industry, we can make better, informed decisions about the genetic health of our colonies.

Inventory and stewardship for pollinators in the Okanagan and Similkameen valleys

Jennifer Heron, B.C. Ministry of Environment, Species Conservation Science Unit, Room 315, 2202 Main Mall, Vancouver, BC Canada V6T 1Z1; email: Jennifer.Heron@gov.bc.ca

British Columbia has the highest bee diversity in Canada (at least 450 species) with approximately one-third of the species within the Western Interior Basin in the south-central area of the province not occurring anywhere else in Canada. Additional pollinator groups, including butterflies and flies, are also diverse and endemic to this region. However, this ecozone also coincides with some of the most desirable real estate in the country, resulting in immense urban, rural and agricultural land-development pressure, combined with threats from livestock overgrazing, wildfire suppression, natural succession, and recreational use. Engaging land managers in stewardship actions for pollinators is challenging, and first involves understanding the species richness and distribution within the area of interest. To meet these objectives, a long-term project to better understand the pollinator (primarily bee and butterfly) fauna in the Western Interior Basin was started in 2010, initially to engage landowners, but also to contribute to national knowledge of the bee diversity in Canada, and to try and prioritize species that are priorities for assessment by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Ultimately, these data will be used to map bee species with plant communities as a means to prioritize sites for pollinator protection. This talk highlights some of the results from those and other invertebrate conservation work.

Symposium abstracts: Urban Insects – They Live Among Us

**Entomological Society of British Columbia
Annual General Meeting**

Pacific Forestry Centre, Victoria, BC, October 15, 2016

Out of the cities and into the forest: Range expansions of non-indigenous introductions in southwest British Columbia

Lee M. Humble, Natural Resources Canada, Canadian Forest Service, Victoria, B.C., Canada

Since surveillance programs were established for the detection of non-indigenous introductions in the mid 1990s, more than 25 introduced species of Coleoptera, Hemiptera, Hymenoptera, and Lepidoptera have been discovered in the urban and managed forests of British Columbia. The life histories and known distributions of selected species are documented and used to infer likely pathways of introduction and to illustrate the importance of anthropogenic influences on their range expansion. Future research needs for species of potential importance to forestry in B.C. are briefly discussed.

Preventing gypsy moth from establishing in British Columbia isn't fun

Tim Ebata, Resource Practices Branch, B.C. Ministry of Forests, Lands and Natural Resource Operations, Victoria, B.C., Canada

My talk outlines how British Columbia has successfully remained "gypsy moth free" and describes some of the difficulties faced in mounting eradication programs in an urban environment.

Treading Carefully on Fire Ants in the Urban Landscape

Rob Higgins, Biological Sciences, Thompson Rivers University, Kamloops, B.C., Canada

Working quietly on ants while sitting in a forest, perhaps a hundred metres from a colleague and a hundred kilometres from the nearest town allows you to develop a specific set of research and social skills. Unfortunately, none of them provide much guidance when you are talking to an angry homeowner who has recently retained a lawyer because of the ants you are studying. Nor guidance when dealing with businesses fearing major losses who need immediate advice you simply aren't sure you have. Nor dealing with the police and fire department that someone has called. Nor needing to keep your data so confidential you cannot share it with your funding agency and certainly not the media who keep asking. Working in a social environment as densely developed as the condominiums you find yourself within is a uniquely challenging situation. Here we will look at the invasive fire ants of BC in the urban landscape and reflect on those times we spent sitting in that quiet forest while stuck in traffic.

180,000 bites later: The aggregation pheromone of the common bed bug is finally identified.

Regine Gries¹, Robert Britton², Michael Holmes² and Gerhard Gries¹, ¹Department of Biological Sciences; ²Chemistry Department, Simon Fraser University, Burnaby, B.C., Canada

Drawing on our 2015 publication in *Angewandte Chemie* (International Edition), the presentation describes our approach to collecting sufficient pheromone sources for identifying the aggregation pheromone of the common bed bug (*Cimex lectularius*;

Hemiptera: Cimicidae), the analytical steps taken to identify the pheromone blend, the pheromone components that mediate attraction and arrestment of bed bugs, and the experiments we have run in the laboratory and in bed bug-infested apartments to test the effect of synthetic pheromone as a trap lure. The presentation also highlights future objectives, including the development of a commercial lure and trap.

Butterfly and moth conservation in urban and semi-urban habitats: Challenges and reflections taken from species at risk recovery projects

Jennifer Heron, British Columbia Ministry of Environment, Vancouver, B.C., Canada

The order Lepidoptera is one of the largest and most studied orders of insects. This group is ecologically and economically important, serving as pollinators of many plants and pests for many others. Butterflies are considered by many to be the most charismatic of the arthropods, and the public enjoys seeing these species in their gardens and surrounding natural environments. Many species of Lepidoptera, especially pollinating groups, are at risk. Although the butterflies are relatively well known, there are many species of moths we know little about, and engaging the public in moth conservation efforts is challenging. In this talk, we summarize the biology of these species and cite examples of Lepidoptera conservation projects and the challenges encountered in urban and semi-urban areas.

Beetles in the City: Carabid diversity in the urban environment

Rob McGregor and Veronica Wahl, Institute of Urban Ecology, Douglas College, New Westminster, B.C., Canada

Ground beetle surveys (Coleoptera: Carabidae) have been widely used to assess habitat quality and the influence of human disturbance on urban, agricultural, and forested landscapes. Here, we describe carabid surveys conducted in urban forest fragments in Coquitlam, B.C., where European carabid species predominate in disturbed forests. In addition, we describe a citizen science program where homeowners and community gardeners trap and identify carabids from urban gardens, in association with insectary plants. Finally, we describe preliminary work to document populations of a threatened tiger beetle, *Omus audouini*, in coastal habitat in Delta, B.C.

Up on the Roof: Surveying Biodiversity in a Unique Urban Landscape

C.G. Ratzlaff and K.M. Needham, Beaty Biodiversity Museum, University of British Columbia, Vancouver, B.C., Canada

We have been conducting a monthly survey of the insects making their home on the "green roof" atop the Vancouver Convention Centre, West. The roof was planted almost a decade ago with 23 species of plants and is nearly six acres in size—the largest green roof in Canada. It is left to grow throughout the year and is mowed only once in the fall. Surrounded by tall buildings and concrete sidewalks, with no significant green spaces nearby, we were curious about which insects might find this "meadow" a suitable habitat. Beginning in April and ending in December, we will have visited the roof once a month, including one black-light trapping event in September, and will have catalogued all of the insects collected there. In this talk, we present our preliminary results and highlight some of the finds we have made to date.

Presentation Abstracts

Entomological Society of British Columbia

Annual General Meeting

Pacific Forestry Centre, Victoria, B.C., October 14, 2016

The uninvited dinner guests... and how to get rid of them

Joshua Pol, Regine Gries and Gerhard Gries, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada

German cockroaches are pests in human dwellings. We hypothesized that German cockroaches are strongly attracted to human foods. In laboratory experiments, we bioassayed the responses of the insects to many food types. In a field experiment, one particularly attractive food type proved as appealing to the cockroaches as the leading commercial bait.

Female black widow spiders respond to semiochemicals from conspecific females

Andreas Fischer¹, Manfred Ayasse², and Maydianne Andrade³, ¹Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada; ²Institute of Evolutionary Ecology and Conservation Genomics University of Ulm, Germany; ³Integrative Behaviour & Neuroscience University of Toronto Scarborough, Canada

Females of many web-building spiders produce long-range semiochemicals (message-bearing chemicals). Males respond to them in their search for females, distinguishing between prospective mates that differ in feeding status (well-fed or starved) and sexual maturity (juvenile or adult).

Female spiders whose webs were destroyed often seek the presence of other females when they rebuild their webs. We tested the hypothesis that females respond to semiochemicals from other females when they select new web-building sites. We ran Y-tube olfactometer experiments with adult virgin females of the Western black widow spider (*Latrodectus hesperus*) and the redback spider (*L. hasselti*), offering them a choice between a blank control stimulus and a treatment stimulus consisting of a conspecific female. We found that *L. hesperus* females avoid sub-adult and adult conspecific females, whereas *L. hasselti* females avoid only adult conspecific females. Females of both species preferred starved to well-fed females. This is the first evidence for semiochemical-guided decision-making by female spiders and for semiochemicals produced by sub-adult females. Future work will focus on the identification of the semiochemicals that mediate the avoidance of well-fed females.

Communication between yellowjacket wasps and symbiotic yeast

Tamara Babcock, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada

Recent studies suggest that yellowjackets may share a mutualistic relationship with fermentative yeast, but little is known about how these organisms find each other. Our research demonstrates that yeast from the digestive tract of yellowjackets produces attractive volatiles when grown on grape juice-infused agar.

Mosquitoes: nectar thieves or pollinators?

Dan Peach and Gerhard Gries, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada

Outside of interactions with humans and pathogens, many aspects of mosquito ecology have severe knowledge gaps. While feeding on floral nectar is important to mosquitoes, they are traditionally thought to not pollinate the flowers they visit. Contradicting this, we report evidence that mosquitoes pollinate common tansy and yarrow.

A song of ants and fire: improving baiting methods for the European fire ant

Danielle Hoefele, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada

The invasive European fire ant (*Myrmica rubra*) defends nests aggressively, rendering gardens, lawns and parks unusable. I tested various foods for foraging activity by European fire ants to determine whether they prefer specific carbohydrates and proteins. These results will be used in the future to develop a more effective insecticidal bait.

Evaluation of two passive horizontal transmission pathways for *Metarhizium brunneum* in *Agriotes obscurus* click beetles

J. P. S. Leung, J. S. Cory, J. T Kabaluk, and A. F. Janmaat, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada

Fungal pathogens are unique among entomopathogens in that ingestion is not required for transmission. They can be passively transferred to conspecifics through direct contact or through contact with contaminated substrates. We discuss the relative importance of these two pathways for *Metarhizium brunneum* in the control of *Agriotes obscurus* click beetles.

Filling in the gaps of the IMD immune pathway of the kissing bug *Rhodnius prolixus*

Nicolas Salcedo and Carl Lowenberger, Department of Biological Sciences, Simon Fraser University

Rhodnius prolixus is a hemathophagous hemipteran vector of the parasite *Trypanosoma cruzi*. Similar to other hemimetabolous insects, the genome of *R. prolixus* had no key components of the highly conserved IMD pathway. However, IMD-related effector immune genes are normally expressed. Using bioinformatics, I propose candidate genes completing the IMD pathway.

Population dynamics of the western tent caterpillar: the roles of fecundity, disease, and temperature

Paul MacDonald, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada

Many populations of forest Lepidoptera exhibit regular periodic cycling in abundance, but mechanisms for such dynamics remain a subject of debate in ecology. I used annual field data (1977–2015) from a cyclical species, the western tent caterpillar (*Malacosoma californicum pluviale*), to elucidate how fecundity, viral disease, and temperature contribute to the cyclical dynamics of five field populations.

A selfish X chromosome in a mushroom-feeding *Drosophila*

Graeme Keais and Steve Perlman, Department of Biology, University of Victoria, Victoria, B.C., Canada

Selfish genetic elements are widespread and powerful forces in evolution. By increasing their transmission relative to the rest of the genome for each generation, they spread rapidly through populations, even if they carry a negative fitness cost. Driving X chromosomes are selfish genetic elements that kill Y-bearing sperm in a number of Dipterans. Because males that carry a driving X transmit almost exclusively X-bearing gametes, they produce predominantly female offspring. We provide the first evidence for a driving X chromosome in a common European mushroom-feeding *Drosophila* species. Males carrying the driving X sire between 80–100% female offspring, and most of their sons (of which there are few) are sterile and appear to lack a Y chromosome. Sperm bundles in driving X males develop abnormally, indicating that the driving X is acting during male gametogenesis.

Photosensitivity in developing mountain pine beetle (*Dendroctonus ponderosae*)

Debra Wertman, Department of Biology, University of Victoria, Victoria, B.C., Canada

This research explores the capacity for photosensitivity in developing mountain pine beetles (*Dendroctonus ponderosae*). The identification of a long-wavelength opsin and negative phototaxis in eyeless beetle larvae, as well as an effect of photoperiod on adult emergence, suggest that light may function in survival and life-cycle coordination in this species.

Earwigs (*Forficula auricularia*) as a biocontrol agent: deciphering a generalist predator

Dennis Quach, Jenny Cory, Tamara Richardson and Margo Moore, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada

The European Earwig (*Forficula auricularia*) has garnered scientific interest and public distrust due to its controversial status as both a beneficial predator and an urban pest. We investigate the potential for its use as a biocontrol agent through dietary gut-content analysis and the effects of temperature on its predation efficacy.

The role of pathogen diversity on the evolution of resistance in an insect

Leon Li, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada

Our objective is to determine whether baculovirus diversity affects the rate and magnitude at which resistance evolves. Using *Trichoplusia ni* as a host, changes in resistance against single versus mixtures of AcMNPV were examined. We found evidence of reduced resistance, as well as increased life-history costs in diverse infections.

Response of epigaeic arthropods to riparian habitat enhancement trials in Kinbasket Reservoir, British Columbia

Charlene M. Wood and Virgil C. Hawkes, LGL Limited Environmental Research Associates

We monitored the response of ground-dwelling spider and beetle assemblages (over 200 species) to habitat enhancement trials in Kinbasket Reservoir, British Columbia, from 2014–2015. Species differed one year post-treatment, with treatment assemblages initially dominated by bare-ground associated species. Monitoring is ongoing to evaluate the turnover in species assemblages as vegetation establishes over time.

Role of toxins in insect defensive symbiosis

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Insects are commonly infected with bacterial endosymbionts that are transmitted primarily from mothers to their offspring, often in the egg cytoplasm. These inherited symbionts play important roles in the ecology and evolution of their hosts, such as protecting them against a wide range of natural enemies, including predators, parasites, and pathogens. Little is known about the mechanism of protection by symbionts. Is there specificity? How do defensive symbionts target enemies without harming their host? We studied protection in a defensive symbiosis between the common mushroom-feeding fly, *Drosophila neotestacea*, and its bacterial endosymbiont, *Spiroplasma*, which protects its host against parasitic nematodes and wasps. We found that *Spiroplasma* encodes ribosome-inactivating proteins, related to Shiga toxins, and that nematode ribosomes show a strong signal of toxin-mediated attack. It is likely that symbiont-encoded toxins are common and versatile tools in defensive symbiosis.

3D images = public understanding*Scott Montague, Blackhole Collections*

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OBITUARY

Thelma Finlayson
(29 June 1914 - 15 September 2016)

Thelma Finlayson passed away September 15, 2016. She was 102 years old, and a beloved teacher, mentor, and colleague. Born on 29 June, 1914, Thelma grew up in Trenton, Ontario. She earned her B.A. (Honours, Biology) from the University of Toronto in 1936, a certification in Taxonomy and Biological Control from ARPE in 1971, and a Doctor of Laws (*Honoris causa*) from Simon Fraser University in 1996. She was named to the Order of Canada in 2005 as “a trailblazing entomologist and a beloved teacher and advisor.” Thelma was a past president of the Entomological Society of BC and an Honorary Member and a Fellow of the Entomological Society of Canada. She was a Life Member of the Entomological Society of British Columbia and a Fellow of the Entomological Society of Ontario. Two insect species, *Anisota finlaysoni* Riotte (Lepidoptera: Saturniidae) and *Mesopolobus finlaysoni* Dogenlar (Hymenoptera: Pteromalidae), have been named in her honour.

Thelma had taken an entomology course from E. M. Walker and began her professional career in 1937 in the Dominion Parasite Laboratory in Belleville, Ontario, eight miles from her home, as a Technical Officer. She obtained her position by patiently sitting in the office of the laboratory until someone needed an extra pair of hands. Even in her early career, she was an implacable force; her first project was to rear “millions of sawflies” (her words) in the newly built quarantine facility, searching for parasites to control European spruce sawfly that were decimating Quebec and New Brunswick forests. This led to her lasting interest in the taxonomy of parasitic larvae. She was, in fact, one of the first women scientists in the federal research service, but in 1946, as a married woman, she was asked to resign because men were returning from the war. However, at that time, her husband Roy had become very ill and would soon be compelled to retire, making it necessary for Thelma to work. She challenged the request to resign by threatening to ensure that every other married woman in the Civil Service would be fired for the same reason. In due course, the Assistant Deputy Minister of Agriculture verified her right to work, saving her own job and establishing an important human rights precedent for the Federal Civil Service. In 1959, she was promoted to Research Officer (levels 1, then 2) and later Research Scientist.

In 1967, Thelma joined seven other scientists, led by Bryan Beirne, who left the Belleville Research Institute for Biological Control to expand the Department of Biological Sciences at the newly established Simon Fraser University, in Burnaby, British Columbia. There, Thelma was appointed Assistant Professor and Curator of Entomology. She helped found SFU’s Pestology Centre, later renamed the Centre for Pest Management, one of the first of its kind. The Department showed its regard for her teaching and research by promoting her to Full Professor in 1976, despite her having only an Honours-level B.Sc.—an unheard-of advancement in the Faculty of Science. She held the position of Professor Emerita from 1979 and was the University’s first Emerita.

While at SFU, she officially mentored seven Masters and Ph.D. candidates, and unofficially, countless others. Graduate and undergraduate students sought Thelma’s advice for decades. In addition to the time she devoted to students, Thelma was an advocate of entomology and education through significant financial contributions toward Pest Management fellowships. Furthermore, she financed an endowment to establish the Finlayson Chair in Biological Control in the Department of Biological Sciences, currently held by Jenny Cory.

Thelma taught courses in introductory biology and, of course, insect biology. The insect biology course was encyclopaedic, and students typically left each lecture with 30 to 40 pages of detailed notes and sketches. This course inspired many biology students to careers in entomology.

In 1971, Thelma was persuaded to be the first advisor for science in the newly formed Academic Advice Centre and, in 1983, she was appointed as Special Advisor, mostly advising students in academic difficulty and retaining that position until 2012. During this time, she advised more than 7,000 students. At age 97, she may well have been the oldest student advisor on the planet. In 2012, the centre was renamed the Thelma Finlayson Centre for Student Engagement.

Thelma's primary interest was in the taxonomy of natural enemies with application to Biological Control. She published approximately 40 papers, memoirs, and book chapters during her career. She was particularly successful in her work on the taxonomy of immature Hymenoptera, which in many ways foreshadowed the use of DNA to identify remains of parasitoids within hosts. She was also consulted on entomological questions by the RCMP, which led to the development and establishment of the internationally recognized Forensic Entomology Laboratory in SFU's Department of Criminology by her faithful friend, Dr Gail Anderson.

In the first lecture of every course Thelma taught, she told students "My door is always open for you." Those who had the privilege of being her students and of being mentored and supported by this singular woman found that statement to be true always.

Written by

BERNIE ROITBERG, DAVE GILLESPIE, and PETER BELTON

Correction:

Gelling, Lea. 2015. Habitat associations of adult Oregon Branded Skipper at Cordova Shore, Vancouver Island, British Columbia. Journal of the Entomological Society of British Columbia 112:57-68.

In Vol. 112 (2015) the species name, *Grindelia oregonia* (Oregon gumweed), is incorrect in the Abstract and Table 3; the correct species is *Grindelia stricta*.d

NOTICE TO CONTRIBUTORS

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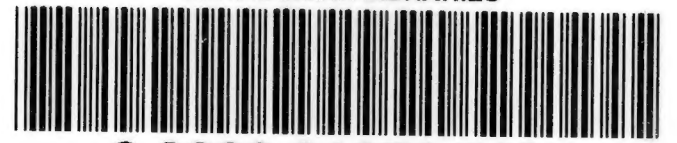
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